VOLUME 15 NUMBER 1

NUTRITION REVIEWS

January 1957

Dietary Fats and Atherosclerosis by Theodore B. Van Itallie 1

Clinical Nutrition
- Obesity in School Children 6
- Fat Digestion in Man 7

Animal Nutrition
- Functions of Cobalt and Copper in Ruminants 8
- Embryonic Malformations in Vitamin E-Deficient Turkeys 9
- Free and "Bound" Niacin for Pigs 11

Experimental Nutrition
- Body Reserves at Low Temperatures 13
- Evaluation of Vitamin B_{12} Assays 14
- Dietary Fat and Liver Fat Deposition 15
- Methionine and Wound Healing 16
- Nature of Dietary Fats and Cholesterol Deposition 17
- Glutamine and Alcohol Consumption 18
- Nutrition in a Parasitic Insect 19
- Vitamin B_6 and Reproduction 20
- Effect of Carbohydrate on Fatty Acid Oxidation 21
- A New Antirachitic Compound 23
- Cortisone and Protein Metabolism 23
- Acetate Metabolism in Diabetes 25
- Spaced Feeding 26
- Sulfate and Molybdenum Toxicity 28
- Potassium and Amino Acids 29

Notes
- Letter to the Editor 31
- A Running Commentary by *The Lancet's* Peripatetic Correspondents 31
- Vitamin B_{12} Activity of Cod Liver Residue 32
- Recent Nutrition Books 32

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT, 1957, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

JEAN M. HOEGGER, One Shattuck Street, Boston

Associate Editors

WILLIAM H. DAUGHADAY, M.D., School of Medicine, Washington University, St. Louis
JAMES S. DINNING, PH.D., Department of Biochemistry, University of Arkansas School of Medicine, Little Rock
R. W. ENGEL, PH.D., Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg
D. M. HEGSTED, PH.D., Department of Nutrition, Harvard School of Public Health, Boston
JAMES F. MEAD, PH.D., Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda
OLOF H. PEARSON, M.D., Sloan-Kettering Institute, Memorial Center, Cornell University Medical College, New York
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
WALLACE R. AYKROYD, M.D., Food and Agriculture Organization
GEORGE R. COWGILL, PH.D., Yale University
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Rockefeller Foundation
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 15 JANUARY 1957 No. 1

DIETARY FATS AND ATHEROSCLEROSIS

Epidemiologic studies reported by A. Keys *et al.* (*Arch. Int. Med.* **93,** *328, (1954)*) and others (A. R. P. Walker and U. B. Arvidsson, *J. Clin. Invest.* **33,** *1358 (1954)*; *Nutrition Reviews* **14,** *321 (1956)*) have suggested that the quantity of fat in the diet may be the most important nongenetic factor affecting the level of cholesterol in the serum of man. Until recently, this concept has been fairly widely accepted, and support for it has come from several quarters. For example, biochemical studies have shown that the biosynthesis of cholesterol proceeds from acetyl coenzyme A, a major intermediate in fat metabolism. By means of carefully controlled dietary experiments performed on human subjects it has been possible to demonstrate that the serum cholesterol level can be lowered by making a substantial reduction in the proportion of fat in the diet (V. A. Wilmot and R. L. Swank, *Am. J. Med. Sci.* **223,** *25 (1952)*; Keys and J. T. Anderson, "The Relationship of the Diet to the Development of Atherosclerosis in Man," *p. 181, in* "Symposium on Atherosclerosis," *Publication No. 338, National Academy of Sciences—National Research Council, Washington, (1954)*). The cholesterol content of the human diet does not appear to affect the serum cholesterol to any important degree (Keys *et al.*, *J. Nutrition* **59,** *39 (1956)*).

Autopsy studies done in countries where the per capita consumption of fat is relatively low have shown that atherosclerosis, particularly of the coronary arteries, occurs in such regions far less frequently than in the United States (J. Higginson and W. J. Pepler, *J. Clin. Invest.* **33,** *1366 (1954)*). Clinically, coronary thrombosis occurs rarely in countries where the daily consumption of dietary fat is 50 g. or less (Keys and Anderson, *loc. cit.*).

At the same time, the belief has grown among many physicians and nutritionists that the alarming increase in the incidence of atherosclerosis in portions of North America and Europe may be etiologically related to a significant increase in the per capita consumption of fat in such areas during the last fifty years. Data on food availability suggest that in the United States approximately one half of this apparent increase in fat intake has come from an increased consumption of fats and oils, including bacon, salt pork and butter, and the other half from dairy products (excluding butter), eggs, meat, poultry and fish (M. B. McCann and M. F. Trulson, *J. Am. Dietet. Assn., in press,* (1957)).

During the last twenty-five years isolated reports on cholesterol metabolism have appeared that have tended to challenge the uncomplicated thesis linking quantity of fat intake to serum cholesterol level. At first it was possible to rationalize such findings as aberrant, chance occurrences, or simply incorrect. However, it is now impossible to ignore the fact that under certain dietary circumstances the serum cholesterol can be strikingly lowered by *increasing* the proportion of fat in the diet. Accordingly, the postulate of Keys relating quantity of fat intake to serum cholesterol level has had to be revised.

It is of interest to review briefly some of the events that have necessitated such a revision. In 1933, R. Schönheimer (*Z. f. klin. Med. 123, 749 (1933)*) reported that by feeding a wholly vegetable diet to a patient with hypercholesteremia he had been able to lower the serum cholesterol markedly. J. Groen, B. K. Tjiong, C. E. Kamminga, and A. F. Willebrands (*Voeding* **13,** *556 (1952)*) demonstrated by means of well-controlled and prolonged experiments that an exclusively vegetable diet can lower the serum cholesterol in man, even if the fat

content remains high. During the same year L. W. Kinsell and associates (*J. Clin. Endocrinol. Metab.* **12,** *909 (1952)*) reported that the ingestion of synthetic diets containing relatively large amounts of vegetable fat consistently results in a significant fall in the level of serum cholesterol and phospholipids. Kinsell's findings were confirmed by E. H. Ahrens, D. H. Blankenhorn, and T. T. Tsaltas (*Proc. Soc. Exp. Biol. Med. 86, 872 (1954)*), J. M. R. Beveridge *et al.* (*J. Nutrition* **56,** *311 (1955)*), and H. Malmros and G. Wigand (*Minnesota Med.* **38,** *864 (1955)*).

When Kinsell *et al.* first reported their results with vegetable fat diets it was not clear whether the cholesterol-lowering effects were due merely to lack of dietary cholesterol (vegetable diets are exceedingly low in cholesterol content), or caused by a positive effect of some unidentified material in the vegetable fat. Sterols related to cholesterol and present in vegetable oils were considered as possibly being responsible since these substances were known to interfere with cholesterol reabsorption from the digestive tract (D. W. Peterson, *Proc. Soc. Exp. Biol. Med.* **78,** *143 (1951)*). However, subsequent studies made it clear that the degree of unsaturation of the plant oils correlated best with their depressing effect on the level of serum cholesterol. Since the polyunsaturated fatty acid content of vegetable fat (principally linoleic acid) plays a major part in determining its iodine number (degree of unsaturation), attention was logically directed toward the influence of the essential fatty acids per se upon serum cholesterol levels. Actually, the evidence implicating linoleic and other essential fatty acids as the important constituents in vegetable oils affecting serum cholesterol levels remains inferential. Nevertheless, many investigators are proceeding on the assumption that linoleic acid (and other essential fatty acids) is, in fact, the crucial component.

The striking changes in serum cholesterol level obtained when diets with a high content of certain plant oils are consumed has stimulated considerable interest in the biochemistry of the so-called essential fatty acids (linoleic acid, linolenic acid and arachidonic acid), their role in nutrition and, in particular, their role in the metabolism of cholesterol. The physiology and biochemistry of the essential fatty acids have been reviewed by H. J. Deuel and R. Reiser (*Vitamins and Hormones* **13,** *29 (1955)*). In a recent brief discussion, H. M. Sinclair *Lancet* **I,** *381 (1956)*) has speculated upon the possibility that the hypercholesteremia of northern Europeans and North Americans may reflect a chronic relative deficiency of essential fatty acids.

The argument runs approximately as follows: Cholesterol in the serum is normally esterified mainly with polyunsaturated fatty acids (F. E. Kelsey and H. E. Longnecker, *J. Biol. Chem.* **139,** *727 (1941)*); when these are deficient in the body, cholesterol tends to esterify with the endogenously manufactured saturated fatty acids. Such esters are "abnormal" and may accumulate in the blood, resulting in hypercholesteremia and, ultimately, atherosclerosis. Phospholipids also normally contain essential fatty acids. A diet high in saturated fat (and cholesterol) increases the body's requirement for essential fatty acids; hence, a high animal fat intake (when low in essential fatty acid content) may induce a conditioned essential fatty acid deficiency. Sinclair argues that in recent years the hydrogenation of vegetable oils has destroyed much of their essential fatty acid content while producing unnatural isomers and analogues of these polyunsaturated fatty acids. Deep-fat frying and other practices also lower the essential fatty acid content of the diet, according to Sinclair.

Sinclair points out that the most important of the essential fatty acids is arachidonic acid, with four double bonds. This substance can be formed in the body from linoleic acid, but vitamin B_6 (pyridoxine) supposedly is needed for this conversion. Hence, a deficiency of this vitamin combined with a diet low in arachidonic acid

might result in a conditioned essential fatty acid deficiency, even if the linoleic acid intake appeared to be adequate.

Deficiency states are characterized by biochemical or anatomic lesions that can usually be reversed when the specific nutrient that is lacking is made available to the body and effectively utilized. Whether hypercholesteremia can be considered a consequence of a biochemical "lesion" induced by a deficiency of essential fatty acids is a question that is exceedingly hypothetical at this time. Much investigative work remains to be done before the relationship, if any, between the nutritional status of the body with respect to essential fatty acids and the level of cholesterol in the serum can be clarified.

Although the mechanism of the effect of vegetable oils on serum cholesterol is still obscure, the fact remains that diets containing appreciable quantities of such oils lower serum cholesterol levels in a reproducible manner by 20 to 30 per cent. Accordingly, the question arises whether it will be possible by consumption of supplementary essential fatty acids to offset the hypercholesteremic effect of the conventional American diet high in saturated fats.

Obviously, such a question will be answerable only when the results of carefully controlled, prolonged clinical experiments become available. B. Bronte-Stewart, L. Eales, A. Atonis, and J. F. Brock (*Lancet* **I,** *521 (1956)*; *Nutrition Reviews* **14,** *327 (1956)*) have performed studies on a few Bantus and Europeans in which various oils and fats were successively added to diets low in fat content (Bantus) or moderate in fat content (Europeans). They found that changes in the serum cholesterol levels coincided with changes in the nature of the fat consumed. One hundred g. of butter and beef drippings isocalorically substituted for olive oil in the diet of the Bantus produced a rise in serum cholesterol level. When olive oil was again administered serum cholesterol levels fell even though the intake of olive oil was increased to 200 g. per day. When 100 g. of sunflower seed oil was added to the diet of Europeans, containing 50 g. per day of fat from animal sources, serum cholesterol levels also tended to fall, and rose promptly after the vegetable oil supplements were discontinued. Highly unsaturated animal oils such as seal oil and pilchard oil had cholesterol-lowering effects similar to those obtained with vegetable oils, while hydrogenated vegetable fat and saturated fractions of vegetable and fish oils caused a rise in serum cholesterol levels.

Unfortunately, the experimental periods of Bronte-Stewart *et al.* were very brief (approximately ten days) and the number of subjects studied very small. Thus, although the results obtained were gratifyingly consistent, judgment as to the significance of the findings must be reserved until the work can be repeated using longer control and experimental periods and more subjects.

According to J. E. Nyrop (*Lancet* **I,** *915 (1956)*), calculations based on the figures of Bronte-Stewart *et al.* (*loc. cit.*) indicate that in a high-fat diet the essential fatty acid content of fats should be above 5 per cent and possibly as high as 15 per cent if an increase of the serum cholesterol level is to be avoided.

Since it has not been proven that the cholesterol-lowering effect of vegetable oils resides in their moiety of essential fatty acids, calculations like those of Nyrop must be considered tentative. However, if such calculations are taken as a basis for formulating a hypothetical "antiatherogenic" diet, it seems clear that palatable diets can be prepared easily that provide a proportion of essential fatty acids within the range recommended by Nyrop. Indeed, if natural foods rich in essential fatty acids were not readily available, diets could be fortified with oils containing a high concentration of linoleic acid (as in the experiments of Bronte-Stewart *et al.*). Moreover, the essential fatty acid content of certain animal fats can be increased markedly by feeding the animals concerned a diet high in vegetable oils. The fat of pigs fed a soybean oil diet may con-

tain as much as 39 per cent linoleate. Presumably, a diet relatively high in fat but containing substantial amounts of essential fatty acids would be more palatable than a diet exceedingly low in fat.

Although it may be possible to reduce the serum cholesterol level substantially by means of diets generously fortified with essential fatty acids, the significance of this hypocholesteremic response is poorly understood at both the metabolic and epidemiologic levels. For every fact that is known about the role of essential fatty acids in metabolism, there are many conjectures. All too many sententious pronouncements have been made that the western world is poisoning itself with an excessive intake of fat, particularly of the hydrogenated variety, and through the consumption of processed foods. Unless such assertions are adequately documented, and they have not been, they serve merely to encourage the food faddists and others who thrive on all reports that tend to discredit civilized diets.

A. Keys (*Lancet* **I,** *576 (1956)*) objected to the concept of atherosclerosis as a form of essential fatty acid deficiency partly on the ground that animals fed diets extremely deficient in essential fatty acids often have increased amounts of cholesterol in the liver but no hypercholesteremia or atheroma. It is true that atheromatous lesions and hypercholesteremia have not been described in essential fatty acid-deficient animals; however, D. Kritchevsky *et al.* (*Am. J. Physiol.* **178,** *30 (1954)*) have demonstrated that the feeding of 9 per cent corn oil (containing approximately 42 per cent linoleic acid) is able to suppress to a marked extent the deposition of cholesterol in the aorta of rabbits consuming a diet containing 3 per cent cholesterol. S. L. Shapiro and L. Freedman (*Ibid.* **181,** *441 (1955)*) have shown that the hypercholesteremic response to cholesterol feeding in rats on a sulfur-deficient protein diet is largely prevented by adding to the diet a fat relatively high in essential fatty acid content. It can be argued that, although an essential fatty acid deficiency by itself does not induce atherosclerosis in the herbivorous animal, in the carnivorous or omnivorous animal (man included) one of the consequences of essential fatty acid deficiency might be an inability to resist the hypercholesteremic effect of a diet high in saturated fat.

Thus, although the relationship between essential fatty acid metabolism and serum cholesterol level remains obscure, there is no *a priori* reason for rejecting Sinclair's hypothesis that nongenetic hypercholesteremia is a manifestation of essential fatty acid deficiency.

The epidemiologic data appear to provide little, if any, support for Sinclair's hypothesis. Availability of fat has increased approximately 24 g. per capita per day in the United States during the last half century ("Consumption of Food in the United States 1909–52," *U. S. Department of Agriculture Handbook No. 62, Sept. 1953*). However, availability of cooking and salad oils (rich in linoleic acid) also has increased during the same period (*Idem.*). Furthermore, the hydrogenated plant oils, which are being consumed in increasing amounts in many margarines and shortenings, are not devoid of linoleic acid.

Although there is no evidence that the absolute intake of essential fatty acids in the diet has decreased, a slight relative decrease in essential fatty acid consumption may have occurred during the last fifty years. Preliminary calculations (McCann and Trulson, *loc. cit.*) indicate that fifty years ago approximately 14.6 per cent of available edible fat was in the form of linoleic acid; currently, this proportion has decreased to 12.7 per cent.

Since the incidence of atherosclerotic cardiovascular disease has increased so strikingly in this country during the last half-century, it is difficult to conceive of the rising heart disease rate being due to the rather small relative decrease in essential fatty acid consumption that may have occurred during the same period. However, it is possible that the "requirement" for essen-

tial fatty acids cannot be thought of in simple arithmetic proportion to the total fat intake.

Unfortunately, it is difficult to obtain reliable information concerning the actual dietary intake of Americans during the last half-century. The statistics published by the Department of Agriculture are accounts of food availability, not consumption. Many foods have not been analyzed for essential fatty acid content, and the influence of changing cooking habits in this country on fat intake is hard to evaluate. For example, it appears that many housewives now cook meat in such a way that much of the fat is lost, and bacon fat, which used to be prized as a cooking vehicle, is now frequently discarded.

In the light of new knowledge about the possible role of essential fatty acids in cholesterol metabolism, it is clear that further epidemiologic information about the nature of the fat intake of various populations is urgently needed. But even before such studies can be initiated efficiently more analytic data concerning the essential fatty acid content of a variety of foods must be obtained.

On the basis of admittedly imperfect and incomplete evidence, it appears that there may not have been any noteworthy decrease in essential fatty acid intake relative to total fat intake in the United States during the last fifty years. Therefore, one alternative to be considered is that some other more subtle dietary factor (or factors) has been at least partly responsible for the pandemic of coronary artery disease that has developed in northern Europe and North America. It is also possible that the data on food intake since 1909 should be considered in terms of other variables such as the per capita caloric expenditure. There is evidence suggesting that the average energy output of Americans has decreased substantially during the last fifty years (J. Mayer, *Physiol. Rev.* **33,** *472 (1953)*).

As Sinclair and others have pointed out, hydrogenation not only lowers the essential fatty acid content of vegetable oils but also is responsible for the creation of unnatural isomers of the essential fatty acids that conceivably could act as "antimetabolites." In this regard, it may be noted that tung oil which is rich in the highly unsaturated but unnatural fatty acid, alpha-elaeostearic acid, causes a marked hypercholesteremia when fed to rats on a diet containing cholesterol and cholic acid. Calorically equivalent amounts of a saturated fat such as coconut oil produce significantly lower cholesterol levels when substituted for tung oil in the same diet (D. M. Hegsted, Proc. Eighth Research Conference, American Meat Institute, University of Chicago, March 22–23, 1956, p. 11). On the other hand, many studies have shown that experimental animals thrive on diets containing large quantities of hydrogenated fat.

Acute experiments involving the feeding of appreciable quantities of hydrogenated oils to human subjects apparently have not induced rises in serum cholesterol in excess of those occurring when comparable amounts of animal fat were administered (Malmros and Wigand, *loc. cit.*, Bronte-Stewart *et al.*, *loc. cit.*). Therefore, until definite evidence becomes available implicating hydrogenated products as carriers of potentially harmful substances, it would seem decidedly premature to condemn them to dietary perdition. Nevertheless, since the possibility has not been excluded that some of the substances formed during hydrogenation may be harmful, their status should be promptly and thoroughly investigated.

It must be admitted that the interesting fact that a high intake of certain vegetable oils can lower serum cholesterol levels remains surrounded by a heavy cloud of conjecture. Although an appealing theoretic case can be made for a possible relationship between essential fatty acid deficiency and hypercholesteremia, the facts simply are not available to prove or disprove the hypothesis. For that matter, the evidence linking hypercholesteremia and atherosclerosis in man is far from satisfactory.

In view of what is presently known about

the influence of certain unsaturated oils on the level of serum cholesterol, what course of action should be taken by physicians and nutritionists while supplementary information is being awaited? Obviously, there can be no ready answer to this question. If a diet high in vegetable oil content can be devised that is more palatable than a low fat diet and yet as effective in lowering the level of serum cholesterol, it would seem reasonable to give such a regimen a trial in patients who were formerly maintained with difficulty on diets low in fat. It should be borne in mind that when diets high in vegetable oil content are prescribed it may become harder to reduce the obese patient's weight, or to maintain his weight at the "desirable" level. Unfortunately, it remains to be satisfactorily demonstrated that a nonvegetarian diet consisting of natural foods and supplemented with vegetable oils can lower the serum cholesterol for an indefinite period of time (J. M. R. Beveridge, W. F. Connell, and G. A. Mayer, *Canad. J. Biochem. Physiol.* **34,** *441 (1956)*). The formula-type diets that are known to be effective do not appear to provide a feasible answer to the problem of lowering the serum cholesterol level.

It would seem that a major objective of nutritional research should now be to assess the long-term effectiveness, in terms of level of serum cholesterol, of regimens that furnish "moderate" quantities of unsaturated fats and oils and still permit selection of a well-balanced diet based on common attractive foods. Such an approach might provide a basis for a practical solution to the problem of lowering the serum cholesterol level in large population groups by nutritional means.

Until the status of the essential fatty acids in relation to cholesterol metabolism is clarified, no judgment can be made concerning the possible desirability of recommending major changes in the cooking and eating habits of a large segment of the world's population. It can only be hoped that experimental evidence adequate to provide a basis for responsible recommendations will be forthcoming before a new crop of ill-advised dietary fads can gain momentum.

THEODORE B. VAN ITALLIE, M.D.
Department of Nutrition
Harvard School of Public Health
Medical Service
Peter Bent Brigham Hospital
Boston

OBESITY IN SCHOOL CHILDREN

There have been few studies concerning the problem of obesity in school children. However, M. L. Johnson, B. S. Burke, and J. Mayer (*Am. J. Clin. Nutrition* **4,** *37 (1956)*; *Nutrition Reviews* **14,** *229 (1956)*) have drawn attention to the relative importance of inactivity and overeating in the energy balance of obese high school girls. An important question arising out of such studies concerns the incidence and prevalence of obesity in children of school age. Some further studies by Johnson, Burke, and Mayer (*Am. J. Clin. Nutrition* **4,** *231 (1956)*) provide the beginnings of an answer to this question.

Although estimates of the incidence and prevalence of obesity in school children have been published, there is a paucity of work on this problem. Johnson *et al.* made their study in two communities within the greater Boston area. School records of 6346 children (2658 boys and 3688 girls) were used as a source of information, and the schools studied seemed to be representative of the public school population of the town as a whole insofar as they included well-to-do as well as poorer sections of the community. Height-weight data were obtained from the school records, and the Wetzel channel position for each child was plotted and used thereafter as the criterion for overweight.

The results obtained indicated that more

than 10 per cent of the children were overweight. Developmentally, there appeared to be two main types of obesity in these children, "persistent obesity" which was present throughout the school record and which occurred in 33.7 per cent of the overweight girls and 47 per cent of the overweight boys, and "late obesity" which was present during the latter half of the school record and which involved 12.7 per cent of the overweight girls and 38 per cent of the overweight boys. It appeared that this latter onset of overweight occurred during the winter months, and Johnson *et al.* consider that this "may be taken as emphasizing the importance of inactivity in the etiology of many cases of obesity." This statement recalls the earlier work of Johnson *et al.* (*see Nutrition Reviews, loc. cit.*) which indicated that inactivity was more important than "overeating" in the development and maintenance of obesity in obese high school girls.

Also of interest in this connection are some studies by J. Mayer, P. Roy, and K. P. Mitra (*Am. J. Clin. Nutrition* **4,** *169 (1956)*) on the relation between caloric intake, body weight, and physical work in a group of 213 mill workers in West Bengal. Here it was found that the caloric intake increases activity only within a certain zone ("normal activity"), and that below that range ("sedentary zone") a decrease in activity is not followed by a decrease in food intake but by an increase not only of food intake but also of body weight. Indeed, Mayer *et al.* suggest that "the fact that mechanized, urbanized modern living may well be pushing an ever greater fraction of the population into the 'sedentary' range may thus be a major factor in the increased incidence of obesity."

As regards their study of obesity in Boston school children, Johnson *et al.* point out several serious limitations of the health records which they used. For example, information concerning maturational data and preschool and postgraduate status was not available, and there were also neither a standardized procedure for the physician's evaluation of the child's nutritional status or continuous evaluation of year-to-year progress, nor an opportunity for observation of the individual child. Other errors in the record consisted of no checks on weights and heights, and weights taken with nonstandardized clothing at various times of the day and not frequently enough over the space of a year. Johnson *et al.* again suggest certain limitations of the Wetzel Grid in their studies. The Grid "physique channels" A (above middle, good-obese), M (medium or middle, good) and B (below middle, good-poor) did not divide the children into three equal groups, and the distribution which was skewed toward the "A" side indicated that more of these children tended to be "stocky" rather than "slender." Johnson *et al.* therefore wonder "if the findings . . . are characteristic only of this particular area and cultural group, or . . . a more general characteristic of growth and development not compensated for by the grid. If this is generally true, the Wetzel Grid would classify a higher proportion of children after 12 years of age as 'stocky or obese' than would be expected if in-channel progress were characteristic."

Despite all these qualifications in the interpretation of their data, Johnson *et al.* have conducted a needed and valuable survey, and one which should stimulate further work along similar lines.

FAT DIGESTION IN MAN

In vitro studies (B. Borgström, *Acta physiol. Scandinav.* **25,** *328 (1952)*; *Acta chem. Scandinav.* **7,** *557 (1953)*) and work with experimental animals (F. H. Mattson, J. H. Benedict, J. B. Martin, and L. W. Beck, *J. Nutrition* **48,** *335 (1952)*; R. Reiser, M. J.

Bryson, M. J. Carr, and K. A. Kuiken, *J. Biol. Chem.* **194,** *131 (1952)*; *Nutrition Reviews* **13,** *25 (1955)*) have indicated that triglycerides are largely hydrolyzed to the monoglyceride stage before absorption. Moreover, the work of Mattson and his coworkers (*loc. cit.*) and of Borgström (*loc. cit.*) has demonstrated that the monoglyceride thus formed in the hydrolysis by pancreatic lipase is the 2-monoglyceride—the 1-monoglyceride isolated in similar studies by many workers has probably resulted from isomerization of the less stable 2-isomer during isolation.

However, it has been shown in some cases that the results of studies of this type may not be completely applicable to human subjects. Furthermore, it is probable that the usual conditions of fat absorption studies using experimental animals, namely the administration of moderately large doses of oil to the fasted animal, may not give an accurate picture of the conditions existing in the case of the normal human diet.

N. H. Kuhrt *et al.* (*J. Am. Oil Chem. Soc.* **29,** *271 (1952)*) isolated and identified 1-monoglycerides from the intestinal contents of 2 human subjects during digestion of fat. D. H. Blankenhorn and E. H. Ahrens (*J. Biol. Chem.* **212,** *69 (1955)*) isolated both monoglycerides and diglycerides from the intestinal contents of 2 human subjects fed a fatty meal, but did not determine in either case whether the diglyceride was the 1,2- or 1,3-isomer or whether the monoglyceride contained the fatty acid in the 1 or 2 position.

In an experiment designed to isolate and characterize the products of partial digestion of fat in man, R. S. Harris, J. W. Chamberlain, and J. H. Benedict (*J. Clin. Invest.* **34,** *685 (1955)*) administered a vegetable oil alone or as part of a meal to several normal subjects. At suitable times after administration of the fat, samples of the intestinal contents were removed through a French Miller-Abbott tube and were carefully extracted and analyzed for fatty acids and 1- and 2-monoglycerides. Although there was considerable variability in the data, it was apparent that the weight percentages of free fatty acids were two to three times those of the monoglycerides and that the 2-isomer usually formed the larger proportion of the monoglyceride fraction. No significant differences were observed in these proportions whether the fat was administered alone or in the meal.

It thus appears from these studies that fat digestion in man follows the same course as demonstrated in *in vitro* studies with pancreatic lipase or as in experimental animals—namely, the hydrolysis of triglycerides to 2-monoglycerides and fatty acids through the 1,2-diglycerides.

FUNCTIONS OF COBALT AND COPPER IN RUMINANTS

It is now well known that several of the diseases reported to affect ruminants pastured on certain types of land are in reality the results of cobalt or copper deficiency (H. R. Marston, *Physiol. Rev.* **32,** *66 (1952)*). However, the mechanisms by which the symptoms are produced have remained obscure. The discovery that cobalt deficiency has its effect in the inhibition of production of vitamin B_{12} by intestinal microorganisms provided some insight into the action of this element (Marston and R. M. Smith, *Nature* **170,** *792 (1952)*; *Nutrition Reviews* **11,** *114 (1953)*).

Using the sheep as the experimental animal, S. H. Allen (*Biochem. J.* **63,** *461 (1956)*) has carried out experiments designed to elucidate some of these problems. Vitamin B_{12} deficiency in sheep was produced on a diet containing less than 0.03 micrograms of cobalt per gram of dry weight. The deficiency symptoms, such as weight loss and decreased

oxygen-carrying capacity of the red blood cells, could be reversed by cobalt solutions given orally or by parenteral administration of the vitamin. Copper-deficient sheep were obtained from flocks which had grazed for thirty months on copper-deficient pastures. Controls for this group were maintained with doses of 10 mg. of copper per day administered thrice weekly as cupric sulfate.

In both groups of deficient animals the concentration of free protoporphyrin in the erythrocytes was markedly increased above the normal, reaching concentrations of up to 500 micrograms per 100 ml. of erythrocytes for the cobalt-deficient and a mean of 171 micrograms per cent for the copper-deficient animals as compared with that of 50 micrograms per cent for both types of controls. In the case of the cobalt-deficient group, administration of 1 mg. of cobalt per day orally, 50 to 100 micrograms of vitamin B_{12} per day by injection, or 20 mg. of pteroylglutamic acid per day intraperitoneally resulted in a gradual remission of the deficiency symptoms and a lowering of the protoporphyrin concentration of the erythrocytes.

The author interprets these results to indicate that in both deficiencies an increased synthesis of protoporphyrin results from the decreased synthesis of heme and a possible general stimulation of hemopoiesis. In the case of the copper deficiency, as has been shown previously (Marston, *loc. cit.*) an iron-deficiency anemia resulted, leading to an overproduction of free protoporphyrin. The cobalt deficiency, however, appears to be complex. In this case, the plasma iron concentration was normal or high and the high concentration of protoporphyrin indicated that vitamin B_{12} is not concerned with the synthesis of this substance. The author feels that these results support the hypothesis that protoporphyrin is not a direct intermediate in heme formation which is evidently markedly inhibited by a deficiency of vitamin B_{12}. This idea has also received support from other experiments (E. I. B. Dresel and J. E. Falk, *Biochem. J.* **63,** *388 (1956)*).

It was evident from these experiments that the deficiency of vitamin B_{12} in ruminants differs markedly from that in man. In the latter case, Addisonian pernicious anemia is accompanied by normal concentrations of free protoporphyrin in the erythrocytes. During remission of the disease following treatment with vitamin B_{12}, the concentration of both protoporphyrin and coproporphyrin rises (C. J. Watson, *Arch. Int. Med.* **86,** *797 (1950)*), possibly because of a general stimulation of hemopoiesis by the vitamin therapy. In the present experiments, no coproporphyrin was detected in the erythrocytes of either normal or vitamin B_{12}-deficient sheep.

EMBRYONIC MALFORMATIONS IN VITAMIN E-DEFICIENT TURKEYS

Reproduction can be uniquely studied in avian eggs where development occurs in a medium apart from the maternal organism. For this reason the fowl is being used to an increasing extent for studying embryonic malformations due to nutritional deficiencies. The turkey hen is known to have a high dietary requirement of nutrients for the production of fertile eggs and healthy poults.

The role of vitamin E in reproduction of turkeys has been studied by R. L. Atkinson, T. M. Ferguson, J. H. Quisenberry, and J. R. Couch (*J. Nutrition* **55,** *387 (1955)*), and by Ferguson, Atkinson, and Couch (*Proc. Soc. Exp. Biol. Med.* **86,** *868(1954)*). In these studies of Atkinson and co-workers a total of 72 turkey hens (30 weeks old) were used. They were divided into eight groups and fed various practical rations composed of corn, milo, soybean meal, necessary minerals and vitamins, including stabilized vitamin A and, in some cases, sources of unidentified factors. Four groups received rations containing no added vitamin E. Another four groups re-

ceived these same rations supplemented with 44 mg. of alpha-tocopheryl acetate per kilogram of ration. After the birds had been fed the rations for nine weeks the alpha-tocopheryl acetate supplement was switched to the other four groups in order to evaluate better the effect of this vitamin on reproduction.

The addition of vitamin E to the four different rations resulted in hatchability of 88 per cent over the first nine-week period as compared with an average of 51.7 per cent for the negative control groups. No effect of vitamin E on egg production or fertility was noted. Analysis of samples of egg yolk from the four groups showed that dietary vitamin E increased the tocopherol content from 200 micrograms to 838 micrograms per yolk. Dried whey and fish solubles as sources of unidentified factors likewise had no effect on egg production or fertility and, unlike vitamin E, had no effect on hatchability.

When the diets were switched, hatchability dropped from 88.0 per cent to 78.7 per cent in eight weeks in the four groups given the basal ration. The hatchability of eggs from hens given vitamin E increased from 51.7 to 68.5 per cent. This again showed the importance of vitamin E supplementation, although the differences were not so marked, perhaps due to storage of vitamin E in the birds previously given this vitamin and to the late stage of the breeding season. The tocopherol content of the yolks was again influenced by dietary vitamin E in the same manner as previously noted.

In the deficient groups during the first nine weeks there were 309 dead embryos out of 652 fertile eggs with a "large number" (unspecified) dying between the twenty-fourth and twenty-eighth day of incubation. (The length of incubation of turkey eggs is twenty-eight days.) Most of the deficient embryos were smaller in size than normal and appeared to be blind. They had a cloudy lens or a cloudy spot under the cornea.

A more detailed account of the eye condition of these deficient embryos has been given by Ferguson and co-workers (*loc. cit.*). Associated with the lens disorder was a hemorrhaged vitreous humor and often a bulging of the cornea and an irregular shaped iris. Preliminary histologic examination of the deficient lens showed degenerative changes throughout the lens, especially in the central and posterior portions. Degenerative changes were also observed in the retina although it is not stated how common this defect was. The authors also observed edematous areas on the neck and back of the head as well as edematous toes. All of these signs of abnormality were prevented by dietary vitamin E.

A similar eye condition was also seen in embryos from eggs obtained from a commercial turkey flock. However, it is somewhat surprising that practical rations without an apparent stress factor such as rancid fat would be low in vitamin E. Although the authors do not discuss this point, it is possible that some unknown "stress" condition affected the vitamin E content of the rations, such as long periods of storage or storage under warm conditions. With fresh rations the condition may not have been seen. In spite of these limitations it is important to know that such a deficiency can be produced in turkey hens fed practical rations.

L. S. Jensen, J. S. Carver, and J. McGinnis (*Proc. Soc. Exp. Biol. Med.* **91,** *386 (1956)*) have also produced increased hatchability in turkey eggs (from 66 to 77 per cent) by adding vitamin E to a practical corn-soybean meal ration containing no apparent "stress" factor. Fertility of eggs also appeared to be improved by vitamin E. The addition of 2 per cent of fish liver oil to the basal ration without vitamin E reduced hatchability to 39 per cent. The further addition of vitamin E or DPPD (diphenylparaphenylenediamine, an antioxidant known to spare vitamin E (*see Nutrition Reviews* **14,** *141 (1956)*) resulted in normal hatchability, although the tocopherol content of the egg remained low. Unfortunately,

no description was given in these studies of any embryonic malformations.

There has been an unconfirmed report of the correction of retrolental fibroplasia (a disorder of the eye tissues seriously impairing vision) in human premature infants by additions of vitamin E to the diet (*Nutrition Reviews* **8,** *116 (1950)*). However, C. A. Smith (*Ibid.* **8,** *353 (1950)*) pointed out that this work in human infants has not been adequately substantiated. More recent evidence also indicates strongly that retrolental fibroplasia in premature infants is due chiefly to excessive oxygen therapy (R. M. Forrester, E. Jefferson, and W. J. Naunton, *Lancet* **II,** *258 (1954)*; N. Ashton, B. Ward, and G. Serpell, *Brit. J. Ophth.* **38,** *385, 397, 433 (1954)*; W. R. Hepner, *Am. J. Dis. Chil.* **88,** *356 (1954)*).

An eye disorder in young vitamin E-deficient rats has been observed by E. C. Callison and E. Orent-Keiles (*Proc. Soc. Exp. Biol. Med.* **76,** *295 (1951)*). It would appear that the conditions in rats and turkeys are similar, but much more research is needed on the subject to see if other animals, including man, are also affected. Research is also needed on the relationship of this syndrome to eye deformities seen in embryos from vitamin A-deficient rats (*Nutrition Reviews* **12,** *348 (1954)*).

These studies add to our knowledge of turkey nutrition as well as to an understanding of the role of vitamin E in reproduction.

FREE AND "BOUND" NIACIN FOR PIGS

Evidence has been presented for the hypothesis that at least part of the niacin in corn and other cereals is present in a combination that makes it unavailable to rats (*Nutrition Reviews* **10,** *105 (1952)*; E. Kodicek, *Rep. Prog. Chem.* **48,** *276 (1951)*).

The question has now been investigated further by E. Kodicek, R. Braude, S. K. Kon, and K. G Mitchell (*Brit. J. Nutrition* **10,** *51 (1956)*) in a study with pigs. Corn was milled into two fractions, "grits," which came mainly from the endosperm and contained little niacin, and "hominy meal plus bran." The second fraction was finely ground and heated under pressure with 0.5 normal sodium hydroxide for forty-five minutes, then neutralized with hydrochloric acid and the whole digest dried in a current of hot air. This processed material was then remixed with "grits" in the proportions obtained from the original milling.

The experimental hogs were made deficient in niacin by feeding them from weaning on a preliminary ration composed, in per cent, of ground white corn 79, pea meal 10.5, casein 5, cod liver oil 3, and minerals 2.5. As individual animals ceased to increase in weight they were transferred to one of three experimental diets, of similar composition to the preliminary diet but with the whole corn replaced by either (I) "grits" and "hominy meal plus bran" reconstituted without further processing, (II) the same (with each pig receiving a daily supplement of 6 mg. of niacin), or (III) "grits" and alkali-treated "hominy meal plus bran" as described above. Despite the omission of salt from the mineral mixture in diet III, this still contained an excess of 1.5 per cent of sodium chloride over the other diets.

Each animal received its experimental diet ad libitum for a period of nine weeks, and the mean growth rates for the 6 pigs receiving each treatment were −0.2 (on diet I), +5.0 (on diet II) and +3.2 (on diet III) pounds per week, with the differences all being statistically significant. Some hogs from each treatment were killed and autopsied at the end of their experimental period. Those that had received the reconstituted corn without a niacin supplement showed the ulcerative gastritis and diffuse enteritis described by H. Chick, T. F. Macrae, A. J. P. Martin, and C. J. Martin (*Biochem. J*

32, *844 (1938)*) as characteristic symptoms of niacin deficiency in the pig; the animals that received either a niacin supplement or the alkali-treated material did not show these pathologic changes.

The authors suggest that the partial relief of the niacin deficiency by alkali treatment of part of the experimental diet may be explained by the parallel chemical changes that result from this procedure.

When assays for niacin were carried out, in the usual way, on extracts obtained by hydrolysis of the test material in strong acid, the "hominy meal plus bran" fraction gave a value of approximately 40 micrograms per gram whether or not it had been subjected to the alkali processing. This was the case both with a microbiologic assay procedure and with a chemical procedure involving reaction with cyanogen bromide and para-aminoacetophenone.

The microbiologic assay, using *Lactobacillus casei* as the test organism, was then repeated with extracts obtained from the test materials under milder conditions by refluxing with dilute (0.1 normal) hydrochloric acid. In earlier studies, K. M. Clegg, E. Kodicek, and S. F. Mistry (*Biochem. J.* **50,** *326 (1952)*) had found that this procedure did, in fact, extract nearly all the niacin from cereals, but in a form that gave only about 20 per cent of the full response with the test organism that could be obtained after drastic hydrolysis of the extract. Applied now to the "hominy meal plus bran" fraction, the extract gave a value of 7 micrograms per gram of niacin, whereas the corresponding value obtained with the alkali-treated material was approximately 40 micrograms per gram.

The authors considered therefore that whereas in the former case the niacin was in the "bound" form previously found by E. Kodicek (*Biochem. J.* **48,** *viii (1951)*) to be unavailable to rats, in the latter case the processing of an alkaline pH had been sufficient to liberate the niacin.

This hypothesis as to the nature of the active material in the two cases was tested further by concentration of the extracts, with special precautions against a build-up in acidity, and the use of a chromatographic procedure already described by E. Kodicek and K. K. Reddi (*Nature* **168,** *475 (1951)*). The concentrated extracts were spotted on paper and chromatograms were run for sixteen hours with water-saturated normal butanol as the solvent. The dried sheets were then exposed to cyanogen bromide vapor and sprayed with para-aminoacetophenone. The alkali-treated material gave one spot which coincided with that of added niacin. The extract from the untreated "hominy meal plus bran" also gave only a single spot, but this moved more slowly than niacin; after acid hydrolysis of the extract this was entirely replaced by another single spot that moved with niacin.

The authors concluded from these findings that there is no substantial amount of free niacin in corn, and that the action of the alkali treatment in liberating the "bound" niacin is sufficient to explain the improved growth of the pigs in the feeding trial that received the processed material. They suggest that the same phenomenon may explain any nutritional superiority of lime-cooked tortillas over other corn preparations.

It may be argued, of course, that the observation of a chemical change in a material that has been processed in such a way that its nutritional properties have been improved is in no way proof that the chemical change is the cause of the change in feeding value. Indeed, in a preliminary communication, W. N. Pearson, J. S. Valenzuela, and S. J. Stempfel (*Fed. Proc.* **15,** *568 (1956)*) have reported that niacin deficiency on a rat diet containing 40 per cent of corn can be prevented by simple boiling of the corn as well as by an alkaline treatment. It is to be expected that further work will soon be carried out to investigate this unexpected finding more fully.

BODY RESERVES AT LOW TEMPERATURES

Evidence concerning the extent and nature of body reserves utilized during exposure of animals to cold has only recently been forthcoming. A common feature in small mammals is a loss in weight, indicating considerable withdrawal of body energy stores. However, E. Pagé and L.-M. Babineau (*Canad. J. Med. Sci.* **31,** *22 (1953)*; *Canad. J. Biochem. Physiol.* **33,** *970 (1955)*; *Nutrition Reviews* **14,** *312 (1956)*) have presented evidence indicating that rats kept up to fifty-eight days at 11° C. actually gain weight on normal diets and that the body water remains constant (72 per cent of the fat-free body weight). Obviously, the problem is not simple and must be defined by several parameters, including species differences, body size, temperature depression, preconditioning and diet.

J. S. Hart and O. Heroux (*Canad. J. Biochem. Physiol.* **34,** *414 (1956)*) have measured the composition of the body reserves of mice (in terms of both weight and energy utilized) when these animals lose weight in a cold environment. In these studies, two groups were acclimated for four weeks at 22° to 24° C. and 6° to 8° C. respectively. A high carbohydrate diet (commercial fox chow) was fed ad libitum. At the end of the acclimation period, analyses were made on half the animals in each group. These served as controls for the remainder, which were exposed to freezing temperatures (the 23° C. mice at −7° C., the 6° C. mice at −13° C.) for four days. Analyses of body composition of the mice included measurements of wet weight (minus gastrointestinal tract), dry weight, ether extractables, fat-free dry weight, nitrogen in fat-free residue, and heats of combustion of ether extract and fat-free residue. Significant differences in body composition of the control animals were observed after warm and cold acclimation. Although the two groups (originally kept at 23° C. and 6° C., respectively) did not differ in wet weights, the 6° C. group contained on the average 1.0 g. more water and 0.87 g. less of ether extractable substances. Fat-free dry weight and nitrogen content were not significantly affected by acclimation at 6° C. The 23° C. animals, when exposed to −7° C. for four days, lost weight amounting to 2.34 g., this loss consisting of 0.72 g. of water, 1.43 g. of ether extractables, and 0.28 g. of protein. When the 6° C.-acclimated group was exposed to −13° C. there was an average weight loss of 5.80 g. made up of 3.01 g. of water, 2.16 g. of ether extractables, and 0.49 g. of protein. Relative to the change in fat-free dry body weight, the water content of the 6° C. group changed more on exposure to cold than did that of the 23° C. group. In the fat-free portions of the carcasses of the 6° C. group the water content dropped from 73 to 71 per cent. In the 23° C. group it remained constant at 72 per cent.

On an energy basis, the body reserve lost during the four-day exposure to freezing temperatures was approximately 15,000 Calories for the 23° C.-acclimated mice and 24,000 calories for the 6° C. mice, or 6000 and 4100 calories per gram of weight loss, respectively. By dry weight the loss amounted to approximately 8400 calories per gram, mainly as fat, for both groups. There was no significant difference between the 6° C. and 23° C. mice in the proportion of total energy lost from fat stores.

Although the cold-acclimated mice (6° C.) lost more body reserves during exposure to −13° C. than did the 23° C.-acclimated mice at −6°C., this may be due to the difference in environmental temperature rather than to physiologic differences between the two groups. Useful controls would have been (1) a group conditioned at 23° C. and exposed to −13° C. and (2) a group conditioned at 6° C. and exposed to −7° C.

The authors feel that the greater water

content of cold-acclimated controls may be associated with greater metabolic activity of the tissues. Increased cellular water has been detected during muscular activity (J. Barcroft and T. Kato, *Tr. Roy. Soc. (London)* **B 207,** *149 (1936)*; W. O. Fenn, D. M. Cobb, J. F. Manery, and W. R. Bloor, *Am. J. Physiol.* **121,** *595 (1938)*) and during exposure to cold (C. Kayser and L. Contcheff, *Trav. membres soc. chim. biol.* **23,** *1229 (1941)*). The failure of Pagé and Babineau (*loc. cit.*) to detect such a phenomenon in rats during exposure to cold may be due to several factors, not the least of which may be the experimental environment of 11° C. rather than 6° C. as employed for the mice in the above study.

Tests on heat of combustion revealed that as the lipid reserves were depleted, the mice first utilized high energy lipids, leaving a residue of lower energy value. An increase in the iodine number of body fat during exposure to cold (G. Clement, *Arch. sci. physiol.* **4,** *5 (1950)*) would probably not result in a sufficiently large decrease in energy to explain the observed effect.

EVALUATION OF VITAMIN B_{12} ASSAYS

Vitamin B_{12} was designated as the name for the compound with antipernicious anemia activity that was isolated in crystalline form in 1948. This compound, like concentrated liver extract, likewise had "animal protein factor" or "factor X" activity for chicks and rats. It also promoted the growth of such microorganisms as *Lactobacillus lactis* Dorner, *L. Leichmannii* and *Euglena gracilis*. When other compounds with like activity came to be known, the term "cobalamins" was suggested for them, with the provision that the original crystalline material, vitamin B_{12}, be called "cyanocobalamin," since it contained a cyano group in its molecule.

Cobalt-containing pigments were then found in natural materials that did not contain the 5,6-dimethylbenzimidazole group, which is characteristic of "cobalamins," so the term "pseudovitamin B_{12}" was proposed. "Pseudovitamin B_{12}," for example, contains adenine instead of 5,6-dimethylbenzimidazole. The "pseudocobalamins" are characteristically inactive for animals but exhibit varying degrees of activity for microorganisms. A further complexity entered when the terms "factors A, B, C, and C_2" appeared in the literature for pseudovitamin B_{12} compounds with activity for certain microorganisms.

This complexity of the cobalt-pigmented class of compounds is pointed out in the report of a recent attempt to evaluate microbiologic and animal (chick) assays for vitamin B_{12} activity in feed supplements and other natural products (W. L. Williams, A. V. Stiffey, and T. H. Jukes, *J. Agr. Food Chem.* **4,** *364 (1956)*).

These workers compared three microbiologic assays (USP *L. leichmannii*, *E. coli* and *Ochromonas malhamensis*) with the chick-growth method. It had been reported earlier that the *Ochromonas* organism failed to respond to all pseudocobalamins which have been detected in natural products (J. E. Ford, *Brit. J. Nutrition* **7,** *299 (1953)*; *Nutrition Reviews* **13,** *185 (1955)*). Although additional pseudocobalamins have been produced by growing *E. coli* on various nitrogen bases and "factor B," some of which are active for *Ochromonas* (Ford, E. S. Holdsworth, and S. K. Kon, *Biochem. J.* **59,** *86 (1955)*; *Nutrition Reviews* **13,** *240 (1955)*), none of these has been found in natural products. Thus, the parallelism between animal response and that of *Ochromonas* appears valid.

The natural products assayed by Williams and associates included fermentation products, concentrates from sludge, vitamin B_{12} feed supplements, fish solubles and liver.

Although the *Ochromonas* assay was in relatively good agreement with the chick growth response, there were notable exceptions. The vitamin B_{12} feed supplement, for example, contained 6.6 mg. of vitamin B_{12} per pound, based on chick assay, while the *Ochromonas* assay indicated 15.6 mg. per pound. Of the 12 fermentation products and sludge concentrates, the average vitamin B_{12} content was 34.1 and 37.2 mg. per pound for chick and *Ochromonas* assays respectively, which would indicate rather uniform response. A mixture of vitamin B_{12} and pseudovitamin B_{12}, 0.5 microgram of each per milliliter, yielded values with the USP *L. leichmannii* and *E. coli* methods of 0.8 and 0.9 micrograms per milliliter respectively, whereas the *Ochromonas* method apparently responded only to the vitamin B_{12} (0.56 micrograms per milliliter).

The authors conclude that although the *L. leichmannii* method will give reliable results with most vitamin B_{12} feed supplements, it would be advisable to make at least a check test with the *Ochromonas* and the chick assay. The pseudocobalamins—*i.e.*, substances which promote the growth of certain microorganisms but not the growth of animals—apparently do not occur to a wide extent in most vitamin B_{12} feed supplements.

DIETARY FAT AND LIVER FAT DEPOSITION

Although it has been convincingly demonstrated that fatty infiltration of the liver occurs in rats on a diet containing only 9 per cent casein even in the presence of adequate amounts of choline and threonine (*Nutrition Reviews* **13,** *217 (1953)*), few experiments have been performed which would adequately assess the effect of the type of fat in the diet on liver fat. Thus H. J. Channon and H. Wilkinson (*Biochem. J.* **30,** *1033 (1936)*) tested the effect of six different fats on liver fat deposition in adult rats but used only olive and cod liver oils as examples of unsaturated fats rather than the common seed fats which contain high percentages of linoleic and linolenic acids. The amount of protein in these diets was only 5 per cent and choline was given at a level of only 1.5 mg. per rat per day. These workers concluded that the infiltration of fat into the liver was proportional to the per cent of saturated C_{14} to C_{18} fatty acids in the fed fat, although they made no attempt to free the fats of components other than the glycerides containing these fatty acids.

In an attempt to clarify this problem, D. A. Benton, A. E. Harper, and C. A. Elvehjem (*J. Biol. Chem.* **218,** *693 (1956)*) designed an experiment with weanling rats, in which the diet contained 9 per cent casein, 0.15 per cent choline chloride and 20 per cent of either butterfat, margarine, lard, corn oil, or the glycerides prepared from the fatty acids of butterfat or corn oil. At the end of two weeks, the rats on the butterfat-containing diet had gained the least weight and the most liver fat. The effect of feeding lard was comparable, while corn oil and margarine promoted the greatest weight gain and the lowest liver fat. The same order was followed when 0.36 per cent threonine was added to the diet, although liver fat was reduced in all animals by this treatment. Increasing the dietary casein level to 18 per cent increased the weight gain and reduced the liver fat to comparable levels with both corn oil or butterfat.

The nonsaponifiable portion of butterfat had no effect on liver fat when fed with corn oil. Further proof that the differences between the two fats lie in the fatty acids themselves was obtained by feeding glycerides prepared from the isolated fatty acids of corn oil or butterfat. Liver fat on these diets was little different from that of the rats fed the complete fats. Reduction of the

casein level to 7 per cent produced an increase in liver lipid which was still further increased by exclusion of choline from the diet.

In order to localize further the effect of the dietary fat on liver fat deposition, the liquid and solid fatty acids from butterfat were fed as triglycerides. The former led to somewhat less and the latter to somewhat greater fatty infiltration than did the butterfat itself. Finally, a synthetic glyceride containing equal parts of oleic and stearic acids, when fed at the 20 per cent level, led to fatty infiltration comparable to that produced by butterfat.

It is evident from these experiments that butterfat has a greater tendency to produce fatty livers than does corn oil but that this tendency becomes apparent only when dietary protein or protein and choline are inadequate. The factors responsible for this difference and for the production of fatty livers seem to be the long-chain saturated fatty acids which are present in greater proportion in butterfat than in corn oil. It has been postulated that the polyunsaturated acids may actually have a lipotropic effect, since fatty livers occur in the fat deficiency state (R. W. Engel, *J. Nutrition* **24,** *175 (1942)*; A. B. Decker, D. L. Fillerup, and J. F. Mead, *Ibid.* **41,** *507 (1950)*).

It seems curious that in these experiments, in contrast to those of H. J. Thomasson (*J. Nutrition* **56,** *455 (1955)*; *Nutrition Reviews* **14,** *305 (1956)*), feeding of butterfat led to lower weight gains in all cases except, possibly, where 9 per cent casein was supplemented with 0.36 per cent threonine and 1.5 per cent glycine. It seems probable that for maximal growth promotion by fat, optimal dietary protein is necessary. Thus, in the experiments of Thomasson 23 per cent protein was used in all diets, whereas in the present experiments the supplemented 9 per cent casein diet or the 18 per cent casein diet may have been barely adequate.

Although the exact nature of the action of the long-chain saturated fatty acids in promoting fatty livers is as yet obscure, it seems probable that an answer may be found in studies concerning the role of the polyunsaturated acids as lipotropic agents.

METHIONINE AND WOUND HEALING

The biochemistry of wound healing has not been extensively correlated with the histochemical changes, and further analysis of this problem is welcome. It has been shown that both protein and methionine favorably modify the pattern of wound healing (*Nutrition Reviews* **11,** *124 (1953)*; **13,** *218, 345 (1955)*) and that the histology of the wound during healing may be correlated with the chemical estimation of mucopolysaccharides and collagen (*Ibid.* **14,** *152 (1956)*). K. N. Udupa, J. F. Woessner, and J. E. Dunphy (*Surg. Gynec. Obstet.* **102,** *639 (1956)*) have continued these studies by examining the effects of methionine or the production of mucopolysaccharides and collagen in the healing wounds of protein-depleted animals.

Young rats were divided into three groups, a normal control, a protein-depleted group, and a protein-depleted group receiving a diet supplemented with 0.147 per cent DL-methionine. A standard wound was studied at three, six, nine and twelve days after surgery.

At three days the protein-depleted animals showed a lesser inflammatory response histologically than did the controls. In accord with this was the observation that there was a smaller production of acid mucopolysaccharides. When methionine supplementation was used, the wounds appeared normal and the hexosamine content was a high normal value.

At six days, the histologic differences between the wounds of the normal and de-

pleted animals were not so striking. Quantitatively, the hexosamine content of wounds in the depleted animals was only slightly lower than normal and the collagen content, as measured by hydroxyproline, was the same as the normal value. Methionine supplementation raised the hexosamine content to high normal values.

At nine days, the hexosamine content of the wounds of the protein-depleted animals reached a peak, whereas the other two groups of animals showed a fall in hexosamine content. Evidently the wounds in the protein-depleted animal were going through the same changes but at a slower rate.

At twelve days, the wounds in the protein-depleted rats were more cellular than those in either the control or the methionine-supplemented groups, and the collagen content was lower. Thus maturity of the wound was again seen to be delayed.

The role of methionine in protecting against protein depletion is only speculative at present. Methionine may act by virtue of contributing its sulfur moiety to sulfated mucopolysaccharides. There may be specific stimulatory effects of methionine in fostering the utilization of endogenous protein or in stimulating fibroblastic activity. Both of these latter hypotheses have experimental backing. The unraveling of these mechanisms will surely lead to greater understanding of the fundamental processes concerned in wound healing.

NATURE OF DIETARY FATS AND CHOLESTEROL DEPOSITION

The most recent report by E. F. Hirsch and R. Nailor (*Arch. Path.* **61,** *469* (*1956*)) continues to develop ideas as to why lipids are deposited in arterial walls. These ideas had their inception in the early work of Hirsch on the composition of the lipids of the human atheromatous plaque (S. Weinhouse and Hirsch, *Ibid.* **30,** *856* (*1940*); Hirsch and Weinhouse, *Physiol. Rev.* **23,** *185* (*1943*)). In the years between the publication of these analytic data on the chemical composition of the lipid of the arterial lesions and the appearance of the present report, Hirsch and his co-workers have developed methods which make it possible to delineate better the lipid spectrum of the blood. They have applied these methods to blood analyses during the clinical or experimental development of atherosclerosis and have compared these analytic data with the composition of the lesions at death, particularly in the experimental animal.

The major technic contributed by this group is a simple method of determining the total fatty acid component of the various fractions of the blood lipids on small quantities of serum (F. C. Bauer and E. F. Hirsch, *Arch. Biochem.* **20,** *242* (*1949*); R. Nailor, Bauer, and Hirsch, *Ibid.* **54,** *201* (*1955*)). With this method Hirsch and co-workers have been able to measure the variations in esterified fatty acid content of the cholesterol, phospholipid and triglyceride fractions of blood. These lipid values have been determined in normal human adults (Bauer and Hirsch, *Ibid.* **23,** *137* (*1949*)), and compared with those in infants and children (Hirsch, L. Carbonaro, A. D. Biggs, and F. L. Phillips, *Am. J. Dis. Child.* **86,** *721* (*1953*)), in patients with diabetes (Hirsch and Carbonaro, *Arch. Int. Med.* **86,** *519* (*1950*); Hirsch, B. P. Phibbs, and Carbonaro, *Ibid.* **91,** *106* (*1953*)), and in rabbits with a variety of disorders (Bauer, G. C. Johnson, Carbonaro, and Hirsch, *Arch. Path.* **51,** *441* (*1951*); Johnson, Bauer, Hirsch, and Carbonaro, *Ibid.* **52,** *115* (*1951*); Bauer, Hirsch, Carbonaro, and Johnson, *Ibid.* **56,** *135* (*1953*)).

With this extensive background of experimental and clinical experience this group has recently introduced the concept that the fatty acids which are liquid at body temperature, such as oleic and linoleic acid,

act as solvents in the blood and tissues for the lipids, including cholesterol, which are solid at body temperature (E. F. Hirsch and R. Nailor, *Arch. Path.* **59,** *419* (*1955*)). In the present study of Hirsch and Nailor (*Ibid.* **61,** *469* (*1956*)) the thesis of the solvent action of the short-chained or unsaturated fatty acids with low melting points has been put to an experimental test. Using rabbits (on a low-fat stock diet) as the experimental animal and cream as a source of a lipid mixture which is fluid at body temperature, these investigators have studied the blood lipid changes occurring in animals fed by stomach tube 25 to 50 ml. of cream daily containing either 1 or 0.5 per cent cholesterol. They have compared the blood lipid values of these animals with those occurring in fasted rabbits or in rabbits given cream alone or cholesterol alone, in addition to the stock ration. In general, they found that rabbits given cream along with dietary cholesterol developed a less severe hyperlipemia and that the blood lipids of these animals contained a higher proportion of neutral fat and phospholipid in relation to the levels of cholesterol and cholesterol esters as compared to the inordinate rise in cholesterol and cholesterol esters observed in the animals receiving only the cholesterol supplement.

When the organs of these various groups of rabbits were studied pathologically it was apparent that the rabbits which had received cream along with the dietary cholesterol had only mild to moderate lesions in their aortas as compared to the severe atherosclerosis exhibited by the rabbits receiving the cholesterol supplements without cream.

Chemical analyses of the aortas of the rabbits receiving cholesterol with and without cream also showed marked contrasts which reflected the contrasts found in the blood lipids. The presence of cream in the daily oral feedings apparently led to deposition of less cholesterol and other lipids in the aortic wall, and there were lower ratios between serum lipids and aortic lipids in the groups fed cholesterol plus cream as compared to the group which had only cholesterol added to the basal diet.

Thus the chemistry of the blood and of the aortas and the histologic findings seem to support the hypothesis that a liberal dietary supply of a low-melting point lipid will decrease the deposition of solid lipids (cholesterol, etc.) in the aortic wall.

It is unfortunate that the quantity of cholesterol fed the group without cream is not stated, so that it is difficult to evaluate whether these animals were given more or less cholesterol than the cream-fed groups.

Nevertheless, the experiments and the ideas behind them are intriguing. They should certainly be repeated in mammals which do not show the peculiar susceptibility to cholesterol feeding characteristic of the rabbit. Since it is becoming more apparent that dietary cholesterol has little influence on hyperlipemia in man (A. Keys *et al.*, *J. Nutrition* **59,** *39* (*1956*)), it would seem important to base experimental conclusions on work in animals which are more comparable to man in this respect.

Perhaps with greater delineation of the food fats which can prevent lipid deposition in the aorta and other arteries and a greater knowledge of the composition of the lipids being deposited, the relative roles of fatty acids, phospholipid and cholesterol in atherosclerosis will become clearer.

GLUTAMINE AND ALCOHOL CONSUMPTION

There is some evidence that nutrition may play a role in alcoholism. J. Mardones (*Quart. J. Studies Alcohol* **12,** *563* (*1951*); *Nutrition Reviews* **11,** *212* (*1953*); **12,** *142* (*1954*)) reviewed some of the effects of diet on voluntary alcohol consumption by rats

and concluded that extra vitamins, particularly thiamine, were effective in reducing alcohol consumption. He also presented evidence that an unidentified factor found in brewer's yeast, liver and other natural materials was effective.

This problem was approached in a different manner by J. M. Ravel *et al.* (*J. Biol. Chem.* **214,** *497 (1955)*) who studied alcohol toxicity in *Streptococcus faecalis*. They noted that in this microorganism the inhibition of growth produced by several alcohols including ethyl alcohol could be reversed by liver extract. Further work led to the isolation of the active material and its identification as glutamine. The addition of adequate quantities of pure glutamine to the culture tubes would completely reverse the growth inhibition produced by ethyl alcohol.

These results were extended to animals by L. L. Rogers, R. B. Pelton, and R. J. Williams (*J. Biol. Chem.* **214,** *503 (1955)*). Rats were housed in individual cages to which were attached two bottles, one containing water and the other 10 per cent ethyl alcohol. The quantity of alcohol consumed by each rat was carefully measured. The diet was a commercial laboratory chow. Rats which consistently consumed large quantities of alcohol were selected to determine the effect of glutamine administration. The oral administration of 100 mg. of glutamine daily reduced alcohol consumption 35 per cent. When the glutamine was discontinued alcohol consumption promptly increased to the former high values. The intraperitoneal administration of glutamine did not affect alcohol consumption.

In a recent paper L. L. Rogers, R. B. Pelton, and R. J. Williams (*J. Biol. Chem.* **220,** *321 (1956)*) have enlarged this study to include other amino acids. The supplements tested were glutamine, glutamic acid, monosodium glutamate, asparagine and glycine. All were fed at a level of 100 mg. per day. Glutamine again reduced alcohol consumption, in this experiment by 34 per cent. The other supplements were completely ineffective; in fact, their administration led to an increase in alcohol consumption.

These results show that under the experimental conditions employed oral glutamine will reduce voluntary alcohol consumption by rats. The extension of these experiments to human alcoholism is the next logical step. Such experiments are difficult because of technical problems, particularly the uncooperative nature of alcoholic subjects. However, such studies have been initiated and hence might permit including sufficient numbers, placebo controls, sufficient time, and other precautions essential for valid conclusions.

NUTRITION IN A PARASITIC INSECT

During past years a number of publications by H. L. House have appeared describing the laboratory propagation of the dipterous insect *Pseudosarcophaga affinis* (blowfly) on artificial diets (*Rep. Quebec Soc. Protection Plants* **31,** *134 (1951)*; *Canad. J. Zool.* **32,** *331, 342, 351, 358 (1955)*). These chemically defined diets were, however, obviously deficient. For example, the growth of larvae was slow, 9.2 days being required to reach the third instar as compared with approximately five days on a pork liver diet.

A recent publication by H. L. House (*Canad. J. Zool.* **34,** *182 (1956)*) describes some improvements in the diet and notes the effect on growth and development of varying concentrations of an amino acid mixture, dextrose, potassium ion, salt mixture and lard or a mixture of fatty acids. The diet which had been developed in previous work was used as the basal medium for these studies. It was compounded in two parts: Part I consisted of a solution of 2 g. of dextrose and the water-soluble vitamins in 20

ml. of water. Part II was a combination of 2.0 g. of a mixture of amino acids, 0.75 g. of salt mixture No. 2, 0.1 g. of ribonucleic acid, 0.75 g. of agar, 10.0 ml. of a mixture of 0.1 g. of cholesterol and 2.2 ml. of 2 normal sodium hydroxide, and 67.8 ml. of water. Larvae were recovered from gravid females, divided among various diets and reared at a constant temperature of 23°C.

It was found that very closely defined proportions of dextrose (0.5 per cent), amino acids (2.5 per cent), salt mixture (0.668 per cent) and potassium ion (0.17 per cent) were required for optimal growth. No experiments were performed, however, in which these proportions were retained while the total concentration was altered. The optimal amount of dextrose satisfied the requirement for most rapid growth in the presence of lard. Growth rate was depressed by the omission of lard or dextrose. Although varying the levels of dextrose and lard resulted in significant differences in growth rate, there was no detectable interaction between these two components as judged by analysis of variance of the mixtures.

A substitute for lard was developed which consisted of the following: 22 per cent palmitic acid, 10 per cent stearic acid, 48 per cent oleic acid, 15 per cent linoleic acid and 5 per cent linolenic acid. Both lard and the fatty acid mixture tended to bring the ratio of male to female flies growing to adulthood nearer to unity (0.75 as compared to 0.5 on the fat-free diet). The requirement for fat was independent of that for carbohydrate and it was apparent that fatty acids have specific roles other than caloric ones in *P. affinis*.

The above studies merit the attention of workers in various aspects of the field of nutrition. From the point of view of the nutritional embryologist they offer a unique insight into the requirements and metabolic pathways of growing cells. Similar nutritional studies on embryos of higher vertebrates are needed.

For the nutritional physiologist, the dipterous larvae represent a system for assay of nutrients which is intermediate between the single cell and the more complex multicellular organisms. The number of reactions is greater and the induced effects of different diets are easier to detect than in single cell organisms. Furthermore, the mechanisms are not prone to interaction, diversion and obscuration as in higher multicellular organisms.

The metabolic roles of the fatty acids in this case appear to be, first, changing the ratios of the surviving sexes, and second, fulfilling requirements for growth other than caloric.

A long and hitherto unsuccessful search has been conducted for microbial tools capable of assaying the metabolic pathways of fats (*see Nutrition Reviews* **14,** *243 (1956)*). It now appears that the dipterous larvae may provide a tool for such assays.

VITAMIN B_6 AND REPRODUCTION

The nutritional requirements during pregnancy have been the subject of many investigations. It has been shown, for example, that protein deprivation leads to reproductive failure in the rat (*Nutrition Reviews* **13,** *152 (1955)*). In view of the well known effects of vitamin B_6 on protein metabolism it is not surprising that deprivation of this vitamin also leads to reproductive difficulties. M. M. Nelson, W. R. Lyons, and H. M. Evans (*Endocrinology* **52,** *585 (1953)*) reported that vitamin B_6 deficiency had a deleterious effect on the course of pregnancy in the rat. Of particular interest was the observation that the administration of estrone and progesterone permitted the maintenance of pregnancy in the pyridoxine-deficient rats.

That these observations may have application to human nutrition is indicated by the

reports demonstrating a vitamin B_6 requirement for man (*Nutrition Reviews* **7,** *246 (1949)*; D. B. Coursin, *J. Am. Med. Assn.* **154,** *406 (1954)*). Further, it has been reported that the vitamin B_6 requirement is increased in pregnant women (*Nutrition Reviews* **11,** *75 (1953)*).

The report of M. L. Ross and R. L. Pike (*J. Nutrition* **58,** *251 (1956)*) is of interest not only because the data reemphasize the importance of vitamin B_6 in reproduction but also because it demonstrates that the diet fed prior to gestation may strongly influence reproduction. In these experiments female rats were given a diet of laboratory chow until regular estrous cycles were established. They were then divided into two groups one of which was continued on the chow diet. The second group was given a purified diet devoid of vitamin B_6 and containing 26 per cent casein and the other vitamins and minerals required by the rat.

The animals were fed these diets for from seven to thirty days and until mating was confirmed. Each group was then subdivided into five groups which were given the basal diet alone and supplemented with 0.5 mg. per cent of desoxypyridoxine, and with 0.4, 0.8, and 1.2 mg. per cent of pyridoxine respectively. This experimental design permitted an evaluation of the effects of vitamin B_6 depletion prior to mating on reproductive performance as well as the effects of vitamin B_6 nutrition during gestation. On the twenty-second day of pregnancy the animals were killed and the young were removed. Observations were made on the size of the litters, the number of resorptions, and fetal weights.

Maternal weight gain was strongly influenced by vitamin B_6. The animals fed the vitamin B_6-deficient diet prior to mating gained less weight during pregnancy than did those fed an adequate diet regardless of the quantity of pyridoxine fed during gestation. Likewise, the animals fed an adequate diet prior to mating also required a dietary supply of pyridoxine during gestation for maximum weight gain.

Nitrogen balance studies were conducted on animals in the third week of pregnancy. Again the state of vitamin B_6 nutrition prior to mating strongly influenced the performance during gestation. The animals depleted of the vitamin before mating retained less nitrogen than did those fed an adequate diet prior to mating. It was also noted that in order to obtain maximum nitrogen retention it was necessary to feed approximately 0.4 mg. per cent of pyridoxine during gestation.

Vitamin B_6 deficiency did not affect the number of live young born. However, fetal weights were influenced by the vitamin B_6 intake. Fetuses of normal weight were obtained only from animals fed adequate pyridoxine both prior to mating and during gestation. The incidence of resorption was affected in a similar manner and was high in animals fed the deficient diet either prior to mating or during gestation.

These data show that normal reproduction in the rat is achieved only when the animal is adequately nourished both prior to mating and during gestation. These results may have important application to obstetric practice and indicate that the feeding of an adequate diet during gestation may not necessarily correct nutritional deficiencies acquired prior to conception.

EFFECT OF CARBOHYDRATE ON FATTY ACID OXIDATION

That fat and carbohydrate are metabolically related has been known for some time. In particular, one of the most obvious effects of carbohydrate on fat oxidation gave rise to the once popular quotation that "fat burns in the fire of carbohydrate." Examina-

tion of the facts behind this statement reveals that it was evidently based on the well known effect of carbohydrate in preventing ketosis. A more modern interpretation would be that this effect is accomplished by channeling of acetyl-coenzyme A formed by fatty acid oxidation in the liver via oxalacetate to complete oxidation via the Krebs cycle rather than to acetoacetate formation.

The antiketogenic effect of carbohydrate may be interpreted in two ways (H. J. Deuel and M. G. Morehouse, *Advances in Carbohydrate Chem.* **2,** *119, see esp. p. 145 (1946)*). The ketolysis theory held that the oxidative disposal of acetoacetic acid was aided by products of carbohydrate oxidation. The antiketogenesis theory, on the other hand, resulted from the belief that the preferential oxidation of carbohydrate spared the rapid oxidation of fat and thus minimized the production of ketone bodies.

Recently, the use of tracer technics in fatty acid studies has made possible a reexamination of this question. Using the expiration of $C^{14}O_2$ (radioactive carbon dioxide) by rats as a measure of the oxidation of C^{14}-labeled injected fatty acids, W. J. Lossow and I. L. Chaikoff (*Arch. Biochem. Biophys.* **57,** *23 (1955)*) studied the sparing of fatty acids of different chain lengths (C_2, C_8, C_{10}, C_{12}, C_{14}, C_{16}) by carbohydrate. Rats which were fasted for twenty-four hours before an injection of triglyceride emulsion (containing fatty acids of chain length C_{12} or C_{16}) or sodium salt (containing all the acids) expired significantly more $C^{14}O_2$ than did those which were fed twelve hours prior to injection and given 6 g. of glucose twenty minutes before the injection. The depression of fatty acid oxidation occurred during the first eight to twelve hours after the injection and was followed by a rise in expired $C^{14}O_2$ to levels higher than those observed with fasted animals. Both the amount of depression and subsequent recovery of fatty acid oxidation were greatest with palmitic acid (or tripalmitin) and decreased with decreasing chain length to acetic acid which did not show the effect.

These results could logically support at least two hypotheses. First, it is well known that fatty acid synthesis is enhanced by normal metabolism of an adequate supply of carbohydrate and depressed by conditions associated with lowered carbohydrate metabolism such as diabetes and starvation (*Nutrition Reviews* **13,** *115 (1955)*). Thus, an apparent sparing of fatty acid could in reality be an increase in fatty acid synthesis from the acetyl-coenzyme A derived from fatty acid oxidation. Second, carbohydrate feeding could, in some manner, depress the initial breakdown of fatty acid with the same result.

A decision as to which of these two possibilities is the actual case was reached by Lossow and Chaikoff (*loc. cit.*) from the results of the following experiment. Fatty acids derived from fed or fasted rats after palmitate-1-C^{14} injection (given as described above) were decarboxylated, and the activity in the carboxyl group was compared with that of the hydrocarbon moiety. It was evident that although there had been a greater total synthesis of C_{16} acids in the fed rat, the greatest effect was in the greater chain-lengthening to C_{18} acids in the fed rat as compared with the fasted rat. However, the increased use of C^{14} in these synthetic reactions accounted for only a small fraction of the difference in expired $C^{14}O_2$ of the animals. Thus, it appeared that the principal action of carbohydrate on palmitic acid oxidation is not to funnel the primary oxidation products of palmitate to fatty acid synthesis but rather to depress the breakdown of palmitate. Presumably this explanation would serve equally well for the effect of the longer chain fatty acids. Since the shorter chain acids are not deposited in tissues to any extent as such, however, it would appear that the conversion of these acids to palmitic acid serves as a means of their storage in tissues and this seems to be enhanced by previous feeding.

Although discovery of a mechanism for the depression by carbohydrate of fatty acid utilization is not immediately forthcoming from these experiments, at least one avenue of approach appears promising. The work of V. P. Dole (*J. Clin. Invest.* **35,** *150 (1956)*) and of R. S. Gordon and A. Cherkes (*Ibid.* **35,** *206 (1956)*; *Nutrition Reviews* **14,** *216 (1956)*) has demonstrated that the plasma content of unesterified fatty acids bears an inverse relationship to that of glucose. The latter workers also noted that following the depression of plasma unesterified fatty acid levels by glucose there was a rise to levels higher than the initial values. If, as now seems probable, the unesterified fatty acids represent a major metabolically active fraction of the body's fatty acids, it is possible further study of the interrelationship between blood glucose and plasma fatty acids may further elucidate the mechanism by which carbohydrate spares fat oxidation.

A NEW ANTIRACHITIC COMPOUND

Although vitamin D_3 has been regarded for some time as the chief naturally occurring antirachitic factor, certain discrepancies in the quantitative measurement of the potencies of natural oils and the amounts of the vitamin they contain have led to the thought that other active compounds may be present. Thus R. G. Booth and S. K. Kon (*Biochem. J.* **27,** *1302 (1933)*) found that only 20 to 25 per cent of the antirachitic activity of butter was in the nonsaponifiable fraction. Recently, Y. Raoul and co-workers (*Compt. rend. Acad. sci. franc.* **241,** *1882 (1955)*) demonstrated that the action of floridin on cholesterol led to the production of seco 9/10-5-cholesten-7-one-3 beta, 10-diol. The ultraviolet absorption characteristics of this compound (maximums at 250 millimicrons (E = 22,000) and 310 millimicrons (E = 2400)) had been previously seen in tuna liver oil, but the compound responsible for them had not been isolated (Raoul *et al.*, *Ibid.* **235,** *1439 (1952)*).

With a knowledge of its properties, Y. Raoul and co-workers (*Bull. Soc. chim. biol.* **38,** *495 (1956)*) have now isolated the previously synthesized compound from red tuna liver oil. Chromatographic fractionation of the oil on alumina led to the isolation of vitamin D_3 esters (300 micrograms per gram) and the new compound, "ketone 250" (1680 micrograms per gram). No free vitamin D_3 was found in this oil.

Although the "ketone 250" was found to possess only one-tenth the anti-rachitic activity of vitamin D_3 for rats and chicks, its concentration in the oil was five to six times that of the vitamin so that its contribution to the total activity of the oil was significant.

Having demonstrated the existence of a new antirachitic compound in natural oils, Raoul and co-workers were able to explain some of the discrepancies in vitamin D analysis. First, the presence of large amounts of vitamin A, which is antagonistic to vitamin D, may depress the antirachitic activity of an oil below the sum of the activities of its components. Second, saponification increases the activity of an oil by liberation of vitamin D_3 alcohol but depresses it by loss of "ketone 250" in the saponifiable fraction.

With a better knowledge of the complexity of the antirachitic activity of natural oils, it may now be possible to extract the total activity or to analyze for it with reasonable accuracy.

CORTISONE AND PROTEIN METABOLISM

As more knowledge has accumulated regarding the dynamic aspects of nitrogen metabolism it has become increasingly difficult to separate and delineate metabolic

effects in terms of the classic concepts of anabolism and catabolism.

Anabolism refers to synthesis occurring either from digested food materials or from compounds present in the metabolic pool as a result of degradation of tissue components. Similarly, catabolism refers to breakdown processes affecting proteins and amino acids. In the case of the latter, diversion may take place either before their incorporation into protein or after their hydrolytic removal therefrom. Catabolism, for example, may be interpreted as diversion of amino acids from protein synthesis by the favoring of their oxidative deamination and conversion to carbohydrate fragments, although some might call this antianabolism. Although these two opposing processes are operating continually and a balance is achieved in the normally functioning organism, situations do occur in which the equilibrium is disturbed. Starvation, nutritional deficiency, wasting disease, or endocrine imbalance may accelerate the catabolic process. In growth, anabolism may be favored or catabolism retarded, whereas supervention of maturity may result in decreased anabolism or hastened catabolism.

The action of cortisone in retarding the growth of young animals or in promoting nitrogen excretion in adult animals has aroused many speculations as to the mechanism by which this inhibition of growth is produced. Some have claimed that the primary effect of cortisone is antianabolic (*Nutrition Reviews* **11,** *184 (1953)*). Others have postulated that the chief mechanism of cortisone action is the catabolism of existing tissue protein. H. D. Hoberman (*Yale J. Biol. Med.* **22,** *341 (1950)*; *Nutrition Reviews* **14,** *279 (1956)*) suggests that both tissue protein and amino acid catabolism are accelerated.

P. R. Cannon, L. E. Frazier, and R. H. Hughes (*Arch. Path.* **61,** *271 (1956)*) have recently re-explored the question of how cortisone produces metabolic alterations. Using both protein-depleted and normal rats they have observed some interesting effects of cortisone on protein metabolism. In their first experiment cortisone action was superimposed on a catabolic state resulting from feeding a nitrogen-free ration. The weight loss and negative balance for nitrogen and potassium which usually follow the feeding of nitrogen-free diets became greater in both normal and depleted rats treated with cortisone. Thus, in the absence of dietary nitrogen, cortisone has a catabolic effect. In a second experiment protein-depleted animals were offered an adequate protein diet over a period of twenty-four days. Complete inhibition of weight gain resulted from the daily administration of 5 mg. of cortisone acetate while the untreated controls were able to gain 45 g. in this same period. Regardless of the terminology, the net effect was a reduction in protein synthesis whether one labels it catabolism or antianabolism. In order to determine whether a deficiency of potassium was responsible for the cortisone inhibition the daily offering of potassium chloride was reduced to 5 mg. in the next experiment. No myocardial lesions indicative of potassium deficiency were found either in this or in a subsequent experiment in which the sodium chloride intake was elevated to impose an even greater strain on the potassium balance of the organism.

By determining potassium and nitrogen balances it was possible, however, to visualize the extent of the cortisone effect during the course of protein repletion. Nitrogen and potassium were absent from the diet for the preliminary period so that the balances for both elements were negative. Inclusion of fibrin and potassium in the ration resulted in positive balances of both potassium and nitrogen in cortisone-treated and untreated animals, but the balances became negative for the animals getting cortisone as the experiment progressed. Thus, although a deficiency of potassium was not

implicated as the cause of growth retardation there was a continued loss as a result of this frankly catabolic state.

The possibility that vitamin B_{12} (cyanocobalamin) becomes depleted or is inadequate in quantity as a result of cortisone treatment was suggested by the experiments of J. Meites and Y. S. L. Feng (*Proc. Soc. Exp. Biol. Med.* **85,** *341 (1954)*). To test this idea 3 or 4 micrograms of the vitamin were mixed in the daily diet of cortisone-treated repleting rats, some of which were permitted to feed ad libitum. Several experiments were performed in which the weight recovery of protein-depleted rats was observed. In all cases cortisone reduced the rate of protein repletion and was uninfluenced by the presence of added vitamin B_{12}.

The increased excretion of various B-vitamins and ascorbic acid resulting from cortisone administration suggested that a shortage of these compounds might play a role in the inhibitory effect of cortisone on repletion. Contrary to what was expected, repletion trials with supplements of both B-vitamins and ascorbic acid did not reverse the growth-retarding influence of cortisone.

Because of the possibility that caloric supplementation was responsible for decreasing the cortisone effect in the experiments of Meites and Feng, repletion experiments were done in which the standard ration containing 3 mg. per day of cortisone acetate was supplemented in three out of four groups by the addition of the 12-calorie equivalent of corn oil, fibrin or dextrin. Analysis of the carcass components of some of the rats killed at the beginning of the experiment gave baselines for evaluating the effects of the eighteen-day feeding regimen. Although the additional calories corrected the cortisone inhibition of weight gain, the analyses revealed that this weight increase was not the result of tissue protein synthesis but was due to increased deposition of fat.

Further studies of this type should do much to clarify the metabolic mechanism of cortisone action.

ACETATE METABOLISM IN DIABETES

In spite of a considerable amount of study the exact metabolic lesion of diabetes has not been determined. The use of alloxan-treated animals has provided a convenient experimental approach to the problem. This chemical appears specifically to produce necrosis of the islets of Langerhans, the pancreatic tissue which produces insulin (*Nutrition Reviews* **2,** *344 (1944)*).

Using rats treated with alloxan, various workers have obtained data which suggest that acetate metabolism is impaired in diabetes. For example, it has been shown that diabetic rats convert glucose to fatty acids at a reduced rate (*Nutrition Reviews* **3,** *213 (1945)*). Other evidence suggests that the entrance of pyruvate into the Krebs tricarboxylic acid cycle is reduced in such animals (F. C. Charalampous and D. M. Hegsted, *J. Biol. Chem.* **180,** *623 (1949)*; *Nutrition Reviews* **13,** *74 (1956)*). In both these processes acetate, in combination with coenzyme A, is an intermediate. These results suggest that either the formation of acetate or its utilization is depressed in diabetes.

C. E. Frohman and J. M. Orten (*J. Biol. Chem.* **216,** *795 (1955)*) have studied this problem by injecting acetate-1-C^{14} into normal and alloxan-diabetic rats. After a short time interval (five to fifteen minutes) the animals were killed and their livers were removed. The various intermediates of the tricarboxylic acid cycle were isolated from these livers by a chromatographic procedure and their concentrations and specific ac-

tivities (C^{14} content) were determined. Onset of diabetes led to a profound reduction in both the concentrations and specific activities of the various Krebs cycle intermediates. These data then indicate that in the diabetic rat the oxidation of acetate via the Krebs cycle is severely depressed.

These results differed from data obtained in an *in vitro* study where C^{14}-labeled acetate was incubated with homogenates of livers from normal and diabetic rats (J. M. Felts, I. L. Chaikoff, and M. J. Osborn, *J. Biol. Chem.* **193,** *557 (1951)*). In the latter experiments livers from diabetic rats oxidized acetate to carbon dioxide at a greater than normal rate. In these *in vitro* experiments the pH of both normal and diabetic liver homogenates was adjusted to the same value by the buffer employed. In contrast, in the intact diabetic animal there may be a reduction in the pH of the tissues as a result of the severe acidosis which accompanies the disease. Thus the possibility existed that the defect in acetate metabolism in the intact diabetic animal was the result of acidosis with a consequent change in tissue pH. To test this possibility C. E. Frohman and J. M. Orten (*Ibid.* **220,** *315 (1956)*) fed normal and diabetic rats purified diets with and without 10 per cent sodium bicarbonate. In addition, since there is some evidence that fructose is better tolerated than is glucose by the diabetic subject, the effect of feeding these carbohydrates to diabetic rats was investigated. It was found that neither the source of carbohydrate nor the feeding of bicarbonate affected the hyperglycemia which accompanies diabetes.

The animals were injected with C^{14}-labeled acetate and the livers were treated as in the previous experiments. Thirty minutes before the animals were killed they were injected subcutaneously with large doses of glucose, fructose, or bicarbonate depending upon their dietary regimen. The administration of fructose did not correct the defect in acetate metabolism in the diabetic rats. Again the entry of acetate into the Krebs cycle was severely depressed. However, bicarbonate administration completely relieved this metabolic block. The concentrations and specific activities of the various Krebs cycle intermediates from diabetic rats treated with bicarbonate were equal to the normal values.

These experiments indicate that at least two metabolic lesions exist in the diabetic rat. One, the defect in acetate metabolism, is corrected by bicarbonate. However, the administration of bicarbonate did not correct the hyperglycemia, which indicates that the diabetic rat suffers from another aberration in carbohydrate metabolism. The mechanism of the bicarbonate effect should be elucidated. While it may be related to pH changes there are other possible explanations.

SPACED FEEDING

The practice of feeding rats for limited portions of the day has been employed by a number of workers (N. Werthessen, *Am. J. Physiol.* **120,** *458 (1937)*; S. B. Barker, *Proc. Soc. Exp. Biol. Med.* **72,** *198 (1949)*; J. Tepperman, J. R. Brobeck, and C. N. H. Long, *Yale J. Biol. Med.* **15,** *855 (1943)*; V. C. Dickerson, Tepperman, and Long, *Ibid.* **15,** *875 (1943)*).

Some rather striking and interesting responses to dietary ingredients given under a spaced feeding as compared with an ad libitum feeding regimen have recently been reported (J. S. Finlayson and C. A. Baumann, *J. Nutrition* **59,** *211 (1956)*). When rations were fed for two hours per day, as little as 5 per cent of urea in the diet produced a considerable growth depression whereas under ad libitum feeding conditions a depression of growth was usually not apparent

until the dietary level of urea exceeded 25 per cent.

As a routine procedure rats were trained for spaced feeding experiments as follows: weanlings, 21 days old, were fed a training diet consisting of alcohol-extracted casein, 20 per cent, corn oil, 5 per cent, Wesson salts, 4 per cent, L-cystine, 0.2 per cent, and cerelose 70.8 per cent. All known vitamins required by the rat were added. After ten days of ad libitum feeding the diet was offered for only two hours daily for two weeks. At this time those rats which had returned to their thirty-one-day weight served as experimental subjects. Under this spaced feeding procedure usually about 40 per cent of the animals failed to return to this body weight level and hence were omitted from further study. In parallel with spaced-fed rats, the same diets were fed ad libitum to 21-day-old weanlings.

Under these conditions 5 per cent of urea in a spaced-fed diet produced a 33 per cent growth depression in contrast with only a 14 per cent depression in growth with a diet containing 20 per cent urea and fed ad libitum. In the case of the spaced-fed diet the daily urea intake averaged 0.4 g. contrasted with an intake of 3.6 g. daily with ad libitum feeding. Thus, rats fed ad libitum were more than nine times as tolerant of dietary urea as were the spaced-fed rats.

What appeared to be critical with respect to urea tolerance was the amount of urea consumed per unit of time. Thus, rats receiving the 5 per cent urea diet and spaced fed, and those receiving a 30 per cent urea diet ad libitum, each averaged 0.2 g. of urea intake per hour. The former suffered essentially the same growth depression (33 per cent) as the latter (37 per cent).

This observation is in harmony with the well-recognized hazard of urea in livestock feeds when not properly dispersed. Urea toxicity is seldom encountered in livestock unless the animals consume excessive quantities in a relatively short period of time.

Finlayson and Baumann also tested the toxicity of several other nitrogenous compounds in diets spaced fed and fed ad libitum. Ten per cent of dietary diammonium citrate produced a 56 per cent growth depression when the diet was spaced fed and only a 27 per cent depression when fed ad libitum. Five per cent of ammonium carbonate produced a growth depression (27 per cent) when fed ad libitum, but when spaced fed it produced an 83 per cent depression in growth. Both ammonium salts produced increases in blood urea nitrogen and the degree of rise was correlated with degree of growth depression.

It was hoped that the spaced feeding technic might reveal sparing effects of antibiotics on dietary proteins. Various protein and antibiotic levels were tested, but in none of the spaced feeding trials was there any evidence of protein-sparing by antibiotics (aureomycin, penicillin, or chloramphenicol). A significant growth stimulation did appear in one of 15 trials where the diet was fed ad libitum, viz., in the case of aureomycin fed with a 12 per cent casein diet.

Spaced feeding studies were extended to other mildly toxic compounds known to depress growth under ad libitum feeding. Growth depression from 5 per cent of glycine in the spaced fed diet was 36 per cent, compared with 55 per cent when glycine was fed at a level of 8 per cent ad libitum. Ethanol (20 per cent in drinking water) was less toxic when spaced fed. Similarly, the carcinogen 3[1]-methyl-4-dimethylaminoazobenzene produced less growth depression when spaced fed than when fed ad libitum. L-leucine, however, was more toxic in spaced feeding than in ad libitum feeding.

Finally, the spaced feeding technic was evaluated as a procedure for determining the biologic value of proteins. Rats were fed the basal 12 per cent casein diet (without added cystine) plus 38 per cent of casein, lactalbumin or alpha-protein of soybeans, added at the expense of cerelose. Biologic values

were calculated from the following formula:

$$\text{biologic value (protein P)} = 73 \times \frac{\text{2-week gain of group fed protein P} - \text{2-week gain of controls}}{\text{2-week gain of group fed 50 per cent casein diet} - \text{2-week gain of controls}}$$

Lactalbumin and alpha-protein of soybeans had biologic values of 84 and 56 respectively, when determined in this way, compared with biologic values of 84 and 59 respectively, as reported for the classic nitrogen balance procedure.

The advantages of the spaced feeding procedure are indicated to be real since only small amounts of the test material would be required for determining the biologic value of proteins. A disadvantage would be the time consumed in training the animals for spaced feeding.

This technic is of particular interest in regard to its application in toxicologic studies. Of particular value would be an assessment of nutritional factors protective against poisons as influenced by feeding technics.

SULFATE AND MOLYBDENUM TOXICITY

Molybdenum may be considered a dietary essential for animals since it is necessary for the elaboration of at least one enzyme, xanthine oxidase (*Nutrition Reviews* **11,** *250 (1953)*). However, abundant evidence indicates that an excessive dietary intake of this element leads to toxic manifestations in the animal. Among the signs of molybdenum toxicity is anemia, and it has been demonstrated that extra dietary copper will partially counteract molybdenum toxicity (*Ibid.* **8,** *239 (1950)*; **12,** *77 (1954)*). Sulfate has also been implicated in molybdenum toxicity with the finding that this ion influenced the effect of molybdenum on liver copper storage (*Ibid.* **13,** *142 (1955)*; **14,** *315 (1956)*).

R. Van Reen (*Arch. Biochem. Biophys.* **53,** *77 (1954)*) and Van Reen and M. A. Williams (*Ibid.* **63,** *1 (1956)*) have studied molybdenum toxicity in rats from the standpoint of liver enzyme alterations. Young rats were fed purified diets to which were added varying amounts of molybdenum. The animals were given extra copper to prevent anemia. It was found that dietary molybdenum concentrations up to 1200 parts per million (p.p.m.) did not influence the activities of cytochrome oxidase, catalase, isocitric dehydrogenase, or DNPase of liver. However, liver alkaline phosphatase activities were found to increase with increasing concentrations of dietary molybdenum. The level of this enzyme in the livers of rats fed 1200 p.p.m. of molybdenum was elevated to approximately four times normal values. The addition of molybdenum *in vitro* to liver homogenates did not influence alkaline phosphatase activities. Also, when liver homogenates from normal and molybdenum-fed animals were mixed and assayed for the enzyme, the value was the average of the two taken separately. These results indicated that the effect of dietary molybdenum on this enzyme was not related to an inhibitor or activator but was due to a change in the concentration of the enzyme.

Growth rate was reduced in the molybdenum-fed animals, rats receiving 1200 p.p.m. growing at less than half the rate of the controls in spite of the copper supplement. The molybdenum-fed animals did not develop anemia, presumably because of the extra copper.

The effect of a supplement of 0.3 per cent methionine on molybdenum toxicity was investigated. The extra methionine improved the growth of the molybdenum fed-animals and reduced the liver alkaline phosphatase levels toward normal.

In view of the positive effects of methionine on molybdenum toxicity the influence of other sulfur compounds was investigated. The addition of approximately equimolar quantities of methionine, cystine, or sodium sulfate to the diet containing 1200 p.p.m. of molybdenum reduced liver alkaline phosphatase levels to approximately the same value. The addition of these various sulfur compounds to diets containing no molybdenum did not affect the alkaline phosphatase activities, indicating that their effect was related to an interaction with molybdenum. Injected sodium sulfate was completely ineffective in alleviating molybdenum toxicity.

Surprisingly enough, molybdenum toxicity affected kidney alkaline phosphatase in exactly the opposite manner from the effect observed in liver. The enzyme was reduced in kidneys from molybdenum-fed rats and the addition of the various sulfur compounds to the diet resulted in an elevation in the activity.

In growth experiments, the feeding of methionine, cystine, sodium sulfate, or sodium thiosulfate improved the growth of rats fed toxic levels of molybdenum.

These results demonstrate that by two criteria, growth and liver alkaline phosphatase alterations, certain sulfur-containing compounds will at least partially alleviate molybdenum toxicity. Since inorganic sulfate was as effective in this regard as was methionine or cystine, it seems likely that the latter two compounds were acting after conversion to inorganic sulfate. Several important questions remain to be answered. Among them are the mechanism of the sulfur effect and the biochemical basis for the alterations in alkaline phosphatase which accompany molybdenum toxicity. In view of the apparent importance of alkaline phosphatase in bone formation, the latter question may be of considerable importance.

POTASSIUM AND AMINO ACIDS

Much of the past work done in an attempt to determine the role of potassium in nutrition has been directed toward the effect of the metal on ionic equilibrium especially within the cells. That there might be some relation between potassium and protein was proposed by E. Muntwyler, G. E. Griffin, and R. L. Arends (*Nutrition Reviews* **13,** *149 (1955)*), who found that the plasma and skeletal muscle electrolytes were not disturbed in rats fed a ration deficient in both potassium and protein. This is in contrast to a dietary deficiency of potassium which leads to a loss of skeletal potassium. The latter is replaced to a certain extent (65 per cent) by sodium. If, as has been assumed, hydrogen ions enter the cells to make up for the loss of potassium not covered by the sodium gain, then a plasma alkalosis should result. There is, however, no indication of alkalosis since the plasma bicarbonate and chloride concentrations remain normal in potassium-deficient rats. To compensate for the potassium deficit would require an increase in the concentration of some basic substance.

The observed relation between potassium and protein led M. Iacobellis, E. Muntwyler, and C. L. Dodgen (*Am. J. Physiol.* **185,** *275 (1956)*) to study the free amino acid patterns of muscle, liver and kidneys of rats maintained on a low potassium, low protein, or a combined protein-potassium-deficient ration. Young adult male rats were kept on these rations for a period of five weeks. At the end of that time the tissue was homogenized and proteins precipitated with tungstic acid. The filtrate was chromatographed on paper. Chromatograms of the protein-free muscle filtrates of the rats fed the low potassium ration indicated the presence of two spots not seen in the control

animals. One of these was identified as lysine and the other as arginine. The filtrate from the normal rats showed the presence of glutamic acid, whereas this was missing from the skeletal muscle of the potassium-deficient rats. The spot corresponding to aspartic acid was decreased in size for the muscles from the deficient rats.

The above results suggest that a potassium deficiency in rats is associated with a gain of lysine and arginine and the loss of glutamic acid and some of the aspartic acid in the cellular fluid of muscles. No studies were made on the amino acid composition of the muscles themselves and at present there is nothing to suggest that any abnormality might be found.

The rats that had been fed the combined potassium- and protein-deficient diet showed no change in the free amino acid patterns in the cellular fluids. However, when these doubly deficient animals were supplemented with protein for six days, thus making them deficient only in potassium, the chromatograms of the muscle filtrates were the same as those seen in the animals on the low potassium diet.

The administration of potassium chloride to the rats maintained on the potassium-deficient diet for thirty-five days produced a restoration to normal in the chromatograms of the muscle filtrates within twenty-four hours.

The kidney and diaphragm muscle of the potassium-deficient rats showed changes comparable to those of the skeletal muscle, with the exception that the changes in glutamic and aspartic acids were not so great. There were, however, no changes in the amino acid patterns in the livers of these rats. Chromatograms of the livers of the rats maintained on the low protein or low protein-low potassium diets differed from those in the other groups since there was no spot corresponding to threonine and a reduction in the size of the spots corresponding to valine and leucine.

Results comparable to the above were secured by R. E. Eckel, C. E. Pope, and J. E. C. Norris (*Arch. Biochem. Biophys.* **52,** *293 (1954)*). They found that in potassium-deficient rats lysine made up from 8 to 40 per cent of the metallic cation deficit in the protein-free filtrate from muscle.

These studies are important in showing that potassium may be related in some way to the maintenance of certain free amino acids in cellular tissue. Perhaps it may be advisable to consider the chick and the guinea pig in any extension of this work. Both of these animals have a high requirement for arginine and, in addition thereto, the guinea pig has a high requirement for potassium. It would be instructive to know whether a potassium deficiency in the chick and guinea pig is associated with disturbances in cellular free amino acids.

NOTES

Letter to the Editor

Dear Sir:

The article "Stannous Fluoride and Tooth Decay" (*Nutrition Reviews* **14,** *197 (1956)*) was read with interest. The review has served to bring together many of the papers concerned with this subject. It was unfortunate, however, that one of the most important papers concerning this subject was overlooked, leading the reviewer to observe erroneously that "in these studies no comment is made about any conscientious effort to eliminate the source of error (bias) . . ." The missing reference (J. C. Muhler, A. W. Radike, W. H. Nebergall, & H. G. Day, *J. Dent. Res.* **33,** *606 (1954)*) describes in detail the precautions taken in all clinical work with stannous fluoride dentifrices at Indiana University. We believe that these precautions were exceptionally effective in eliminating error and possible bias.

In addition we believe two papers not mentioned in your review deserve consideration: (1) the Canadian study conducted by H. R. McLaren and H. K. Brown (*Canad. J. Pub. Health* **46,** *387 (1955)*) and (2) the study of A. B. Slack (*J. Dent. Res.* **34,** *785 (1955)*).

More pertinent to the subject is the report given by Gish *et al.* at the thirty-fourth Annual Meeting of the International Association for Dental Research (1956) which indicated that one application of an 8 per cent solution of stannous fluoride is significantly more effective in reducing the incidence of dental caries in children than four applications of the conventional 2 per cent solution of sodium fluoride.

JOSEPH C. MUHLER, D.D.S., PH.D.
Associate Professor, School of Dentistry & Department of Chemistry
Indiana University
Bloomington, Indiana

Editorial Comment: The space available for each article in *Nutrition Reviews* does not permit exhaustive coverage of each subject. Indeed, the original and continuing purpose of the journal has been to summarize and criticize current literature for all those interested in the broad aspects of nutrition. Our review was prepared for publication prior to the March 1956 meeting of the International Association for Dental Research. In addition, the Editorial Board cannot cover orally presented material in *Nutrition Reviews.*

In both research reports by J. C. Muhler, A. W. Radike, W. H. Nebergall, and H. G. Day (*J. Am. Dent. Assn.* **50,** *163 (1955)*; *J. Dent. Res.* **35,** *49 (1956)*), it was stated without further elaboration that the methods used were identical to those in their earlier work (Muhler, Radike, Nebergall, and Day, *Ibid.* **33,** *606 (1954)*). Where the entire validity of the case depends upon these methods, a sentence or two about the specific precautions seems to be merited in each paper, in addition to a numerical reference to a previous publication.

A Running Commentary by *The Lancet's* Peripatetic Correspondents

Once upon a time there was a very poor country, where nobody had enough to eat and the average expectation of life was 24 years. There was also a very rich country, where everybody had plenty to eat and the average expectation of life was 64 years. In the very rich country people used to save up milk and butter and cream and eggs and send them to the very poor country, where they were distributed, especially to the children, who would otherwise have had none. In this way the expectation of life in the very poor country was raised from 24 to 27 years. Meanwhile the expectation of life in the very rich country was rising too, and went up from 64 to 67 years, and everyone who didn't die of cancer of the

lung from smoking too many cigarettes died of coronary thrombosis. Then someone discovered that coronary thrombosis was due to eating and drinking too much milk and butter and cream and eggs. So people stopped eating and drinking milk and butter and cream and eggs in the very rich country, and sent it all to the very poor country, so that the expectation of life in the very poor country might be raised high enough for them to start dying of coronary thrombosis so that they, too, could stop eating and drinking milk and butter and cream and eggs. (*Lancet* **I,** *635 (1956)*).

Vitamin B_{12} Activity of Cod Liver Residue

The oil-free residue of cod liver represents a readily available source of protein and B-vitamins. B. Truscott and P. L. Hoogland (*Canad. J. Biochem. Physiol.* **34,** *191 (1956)*) have recently investigated the nature of the vitamin B_{12} activity of this residue, which has about the same vitamin B_{12} content as does dried beef liver.

Microbiologic assay with *Lactobacillus leichmannii* before and after alkali treatment of the dried defatted liver revealed that about 75 per cent of the activity resided in the alkali-labile cobalamines. The alkali-stable material was fractionated by solvent partition and countercurrent distribution. Subsequent chromatography on starch and paper demonstrated the presence of at least five other components. These were identified by R_f values and ultraviolet spectrums as purine and pyrimidine derivatives. Comparison of the R_f values with those of known compounds of this class indicated that the new products were the desoxyribosides of thymine, guanine, uracil, xanthine and hypoxanthine. The latter three compounds were considered to be artifacts formed by deamination and oxidation of the naturally occurring compounds during preparation of the liver residue.

The authors noted that of all the known purine and pyrimidine derivatives tested for vitamin B_{12} activity, only the desoxyribonucleosides could replace the vitamin as growth factors for *L. leichmannii*. A low activity of the desoxyribonucleotides was shown to stem from their slow hydrolysis to the nucleosides.

It can be concluded that the cod liver residue could replace beef liver as a source of vitamin B_{12} for the pharmaceutical and feed manufacturing industries since the two are similar both qualitatively and quantitatively.

Recent Nutrition Books

Health, Culture and Community. Case Studies of Public Reactions to Health Programs. Edited by Benjamin D. Paul. Russell Sage Foundation, 505 Park Avenue, New York 22, N. Y. Price: $5.00.

Present Knowledge in Nutrition. 2d edition. Prepared from articles published in the journal *Nutrition Reviews* and revised and edited by its Editorial Staff and Advisory Board, 1956. Copies are available from The Nutrition Foundation, Inc., 99 Park Avenue, New York 16, N. Y. Pp. 130. Price: $2.00.

Food Poisoning. 3d edition, revised. G. M. Dack. The University of Chicago Press, Chicago. Pp. 234. Price: $6.00.

A Summary of the Symposium on Inorganic Metabolism: Function of Metallo-Flavoproteins. Bentley Glass. McCollum-Pratt Institute, The Johns Hopkins University, Baltimore 18, Maryland. Pp. 63. This bulletin is a summary of the discussions and formal papers presented at a symposium on Inorganic Nitrogen Metabolism sponsored by the McCollum-Pratt Institute of The Johns Hopkins University on June 21, 22 and 23, 1955. The entire symposium including the discussions and summary is being published in book form by The Johns Hopkins Press.

Cerebral Palsy. Advances in Understanding and Care. Viola E. Cardwell. Association for the Aid of Crippled Children, 1956. Price: $5.00.

BOARD OF TRUSTEES

JOSEPH F. ABBOTT, *Chairman of the Board, American Sugar Refining Co.*

A. C. BENJAMIN, *President, Junket Brand Foods, Chr. Hansen's Laboratory, Inc.*

*F. G. BOUDREAU, *President, Milbank Memorial Fund*

CHARLES S. BRIDGES, *President, Libby, McNeill & Libby*

G. BUITONI, *President, Buitoni Foods Corp.*

*LEROY E. BURNEY, *Surgeon General, U. S. Public Health Service*

*CASON J. CALLAWAY, *Hamilton, Georgia*

*O. C. CARMICHAEL, *President, University of Alabama*

THOMAS P. CARNEY, *Vice President, Research, Development & Control, Eli Lilly and Company*

GEORGE H. COFFERS, *President, National Biscuit Company*

E. B. COSGROVE, *Chairman of the Board, Green Giant Company*

JOHN N. CURLETT, *President, McCormick & Co., Inc.*

WESLEY M. DIXON, *President, Container Corporation of America*

ROGER DRACKETT, *President, Drackett Company*

*LEE A. DUBRIDGE, *President, California Institute of Technology*

*CHARLES WESLEY DUNN, *New York, New York*

J. S. ELLITHORP, JR., *President, Beech-Nut Life Savers, Inc.*

THOMAS C. FOGARTY, *President, Continental Can Co., Inc.*

EDGAR J. FORIO, *Vice President, The Coca-Cola Company*

*CLARENCE FRANCIS, *New York, New York*

DANIEL F. GERBER, *President, Gerber Products Co.*

HENRY J. HEINS, II, *President, H. J. Heinz Co.*

*REV. THEODORE M. HESBURGH, C.S.C., *President, University of Notre Dame*

FRANK C. HILDEBRAND, *Vice President, General Mills, Inc.*

*HERMAN E. HILLEBOE, *Commissioner of Health, State of New York*

JOHN HOLMES, *Chairman of the Board, Swift & Company; President, The Nutrition Foundation, Inc.*

J. M. KAPLAN, *President, Welch Grape Juice Company, Inc.*

RIDGWAY KENNEDY, JR., *Chairman, Abbotts Dairies, Inc.*

*JAMES R. KILLIAN, JR., *President, Massachusetts Institute of Technology; Chairman of Board of Trustees, The Nutrition Foundation, Inc.*

*CHARLES GLEN KING, *Executive Director, The Nutrition Foundation, Inc.*

*GRAYSON L. KIRK, *President, Columbia University*

JAMES E. KNOX, *Chairman, Chas. B. Knox Gelatine Co., Inc.*

R. NEWTON LAUGHLIN, *President, Continental Baking Co.*

J. PRESTON LEVIS, *Chairman, Owens-Illinois*

R. G. LUCAS, *President, California Packing Corp.*

W. F. MCLEAN, *President, Canada Packers, Limited*

WILLIAM H. MCLEAN, *President of Chemical Division, Merck & Co.*

HANFORD MAIN, *President, Sunshine Biscuits, Inc.*

ARMAND MAY, *Chairman, American Lecithin Co., Inc.*

OSCAR MAYER, *Chairman of the Board, Oscar Mayer & Co.*

JOHN T. MENZIES, *President, The Crosse & Blackwell Co.*

JOEL S. MITCHELL, *President, Standard Brands, Inc.*

CHARLES G. MORTIMER, *President, General Foods Corporation*

MILTON C. MUMFORD, *Executive Vice President, Lever Brothers Co.*

W. B. MURPHY, *President, Campbell Soup Co.*

H. EARLE MUSSY, *Vice Chairman, The Quaker Oats Co.*

JOHN C. NAYLOR, *Executive Vice President, Pet Milk Company*

HORACE L. SIPPLE, *Secretary*

*THOMAS PARRAN, *Dean, Graduate School of Public Health, University of Pittsburgh*

RUSSELL G. PARTRIDGE, *Representing United Fruit Company*

PHILIP W. PILLSBURY, *Chairman, Pillsbury Mills, Inc.*

J. G. PLEASANTS, *Vice-President in charge of Research and Development, The Procter & Gamble Company*

L. RATZESBERGER, JR., *President, The Illinois Canning Co.*

ERNEST W. REID, *Chairman, Corn Products Refining Co.*

B. E. RICHMOND, *Executive Vice President, Richmond-Chase Company*

LLOYD E. RIGLER, *President, Adolph's, Ltd.*

ROBERT B. SCHNERING, *President, Curtiss Candy Company*

MILTON L. SELBY, *President, Safeway Stores, Inc.*

ELBERT M. SHELTON, *Secretary, The Baker Laboratories, Inc.*

WALTER SILBERBACK, *President, American Home Foods, Inc.*

*SIDNEY EARLE SMITH, *President, University of Toronto*

*FRANKLYN BLISS SNYDER, *Evanston, Illinois*

FREDERICK W. SPECHT, *President, Armour & Company*

ALFRED N. STEELE, *Chairman, Pepsi-Cola Company*

W. C. STOLK, *President, American Can Company*

VERNON STOUFFER, *President, The Stouffer Corporation*

L. A. VAN BOMEL, *Chairman, National Dairy Products Corp.*

W. H. VANDERPLOEG, *President, The Kellogg Company*

F. R. WILCOX, *General Manager, Sunkist Growers*

R. D. L. WIRTH, *President, Red Star Yeast and Products Co.*

H. J. WOLFLIEBERG, *President, The Nestle Company, Inc.*

C. I. WOOD, *President, Thomas J. Lipton, Inc.*

HARRY W. ZINSMASTER, *President, Zinsmaster Baking Co.*

---

* Representative of the public.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.